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CHANGES IN THE TENSIONS OF CO₂ AND O₂ IN
GASES INJECTED UNDER THE SKIN AND INTO THE
ABDOMINAL CAVITY. BY J. ARGYLL CAMPBELL.

(From the National Institute for Medical Research, Hampstead.)

DAVY(1) in 1823 was the first to inject gas into an animal—thoracic cavity—to study gas tensions therein. Although many subsequent researches have been carried out in which gas was injected into the thoracic and abdominal cavities, very little work has been done on gas tensions in the subcutaneous region and very little research on experimental alterations in gas tensions in any region. Leconte and Demarquay(2) in 1859 injected gas under the skin to determine tensions in this region; their research, obviously handicapped by technique, did not extend beyond recording figures for ordinary conditions. Henderson and Haggard(3) in 1919 studied the changes produced in tensions in gas in the abdominal cavity of dogs under local anaesthesia, during experimental acidosis; they followed the changes in tensions for only a few hours after the injection of gas. Their research is the only one I can find, dealing with experimental alterations in gas tensions. During the past eighteen months I have employed a simpler and handier method to study experimental alterations in gas tensions within the body. Some results have been published (1, 5, 6, 7). In the present paper the technique is described and further observations are recorded.

Method. Gas was injected under the skin of the back of a rabbit or a cat without an anaesthetic. The apparatus employed is shown in Fig. 1. A modified hypodermic needle was inserted into the skin, usually just beyond the point where the back begins to slope downwards towards the tail, the puncture being made anywhere near the middle line. The hypodermic needle was modified to reduce its air space; the socket for the nozzle of the syringe was broken off, leaving only the needle, the blunt end of which was mounted in a small cylinder of solid rubber. A short piece of rubber tubing was then slipped on over the rubber cylinder. The reason for the reduction of air space in the needle was to lessen the risk of contamination with outside air during the withdrawal of gas samples. When gas was being injected into a rabbit, the animal was placed in a box of such a size that it could not turn round; it was

about 50 mm. Hg in about 10 hours and then fell slowly to between 20 and 30 mm. taking between $1\frac{1}{2}$ to 3 days to reach this level at which it remained fairly constant as a rule, until all the gas was absorbed. It is well known that CO_2 diffuses more rapidly than O_2 , but one would have expected the O_2 -tension to fall to its final equilibrium level in a shorter time than two to three days. The constancy of the CO_2 - and O_2 -tensions over long periods is shown in Fig. 2 a, which gives observations extending over 20 days.

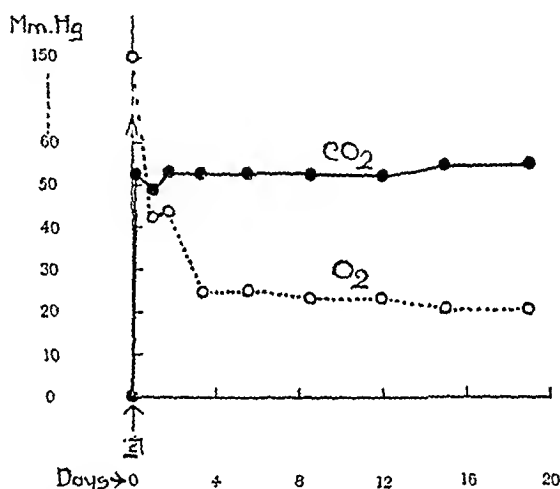


Fig. 2 a. CO_2 - and O_2 -tensions in gas under the skin (rabbit) for twenty days following the subcutaneous injection of atmospheric air.

The rapid rise of CO_2 -tension from 0 to about 50 mm. Hg must have increased the volume of gas under the skin by 6 to 7 p.c. owing to the passage of the CO_2 out from the tissues; this phenomenon has already been observed by others in the case of the thoracic and abdominal cavities.

The curve of O_2 -tension may be divided into three successive periods (cp. Fig. 2 a). In the first there is a rapid fall of tension; in the second there is a slow fall; in the third the tension is practically constant. The first period lasts as a rule 7-10 hours, during which the tension falls from 150 mm. Hg to about 50 mm. Hg. The second period lasts for $1\frac{1}{2}$ - $2\frac{1}{2}$ days, during which the tension falls to about 20-30 mm. Hg. The third period lasts from the end of the second or third day until the whole of the gas is absorbed and during this time the O_2 -tension remains at about 20-30 mm. Hg.

In Fig. 2 b the curves from another animal are spaced to illustrate

in greater detail the changes in tensions for the first five days. In this case the period of slow fall of O_2 -tension lasted at least 60 hours, that is

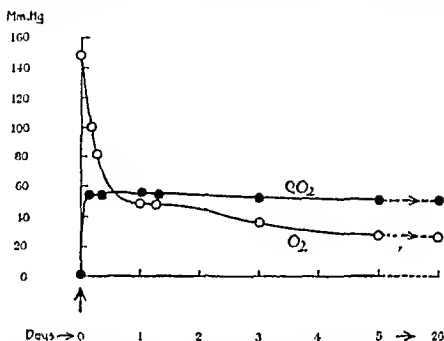


Fig. 2 b. CO_2 - and O_2 -tensions in gas under the skin (rabbit) for the first five days and the twenty sixth day following the injection of atmospheric air.

rather longer than in most animals. The three periods merged gradually into one another, their differentiation not being so clear as when spaced as in Fig. 2 a.

The subcutaneous injection of N_2 . A rabbit not previously injected with gas, was used, the N_2 injected containing no CO_2 and only 0.4 p.e. of O_2 . The results are illustrated in Fig. 3. It will be seen that the

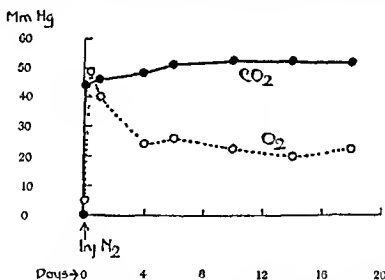


Fig. 3. CO_2 - and O_2 -tensions in gas under the skin (rabbit) for eighteen days after the subcutaneous injection of nitrogen.

changes in CO_2 -tension were similar to those with air. The O_2 -tension, as would be expected, at first increased. But the striking point was that

the tension increased to 49 mm. Hg, *i.e.* to the tension which existed at the end of the quick fall when air was injected. From this stage the tension took the same course as when air was injected, *i.e.* there was a slow fall to 20-30 mm.; this O_2 -tension was permanent till all the gas was absorbed—about 3 weeks. Fuller details of the changes in O_2 -tension on the first day are given in Table I. It will be seen that the O_2 -tension

TABLE I.

Time since N_2 was injected under skin	Tension under skin mm. Hg	
	CO_2	O_2
55 mins.	41	26
2 hrs. 25 mins.	45	38
4 hrs. 20 mins.	45	49
22 hrs. 25 mins.	46	40

rose from 0 to 26 mm. Hg in 55 mins., to 49 mm. in about 4 hours and that it was on the decline by the twenty-second hour after the injection. In my next experiment in the place of pure N_2 I injected a mixture of gases with about those tensions which were found in most animals during the period of constancy of O_2 -tension, that is, CO_2 -tension of 42 mm. Hg and O_2 -tension of 20 mm. Hg.

Subcutaneous injection of gas having a CO_2 -tension of 42 mm. and O_2 -tension of 20 mm. Hg. This mixture was used since the tensions of CO_2 and of O_2 in it were similar to those in the final stage of the previous experiment. The O_2 -tension at first rose (to 52 mm. Hg, *cp.* Fig. 4) as when N_2 only was injected; and was followed as in other cases

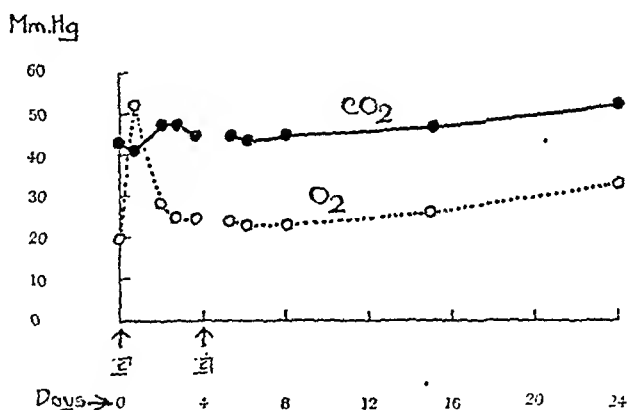


Fig. 4. CO_2 - and O_2 -tensions in gas under the skin (rabbit) for four days following the subcutaneous injection of a small amount of a mixture of gases with tensions, $CO_2=42$ mm. Hg, $O_2=20$ mm. Hg. On the fourth day a larger quantity of atmospheric air was injected and the changes in tensions followed for another twenty days.

by a slow fall (to about 24 mm.). In this experiment a smaller quantity (300 c.c.) of gas was injected, showing—as other experiments showed—that the result is independent of the amount. Further, in this experiment a second injection, this time of air, was made on the fourth day; a sample of gas taken $1\frac{1}{2}$ days later (cp. Fig. 4) showed that the O_2 -tension was unaltered, *i.e.* the final state of constant O_2 -tension had been rapidly attained. In other experiments it was found that when a second injection of air was made during the period of constant tension, the period of slow fall of tension was greatly curtailed.

It will be seen that the O_2 -tension rose slowly and slightly from the eighth day onwards; this will be referred to later.

Subcutaneous and intra-abdominal injection of N_2 . It seemed possible that the slow fall of O_2 -tension in the second period was due to tearing of tissues and consequent temporary vaso-dilation. In order to test this possibility, N_2 was injected into the abdominal cavity and for comparison also under the skin. The results are given in Table II. It will be seen

TABLE II. Rabbit G. N_2 injected under skin and into abdominal cavity.

Time since N_2 was injected	Tensions under skin mm. Hg		Tensions in abdominal cavity mm. Hg	
	CO_2	O_2	CO_2	O_2
17 hours	42	57	42	56
1 day 23 hours	42	43	41	51
3 days	42	34	42	45
4 days	42	30	42	40
4 days 17 hours	42	30	42	40
5 days 18 hours	43	28	43	39

that the changes in O_2 -tension in the abdominal cavity were of the same type as those under the skin. There was a preliminary rise followed by a slow fall. The type of change was the same whether the amount of gas injected was small (200 c.c.) or large (600 c.c.). The former amount would certainly not cause any tearing of tissue in the abdominal cavity of a starved rabbit and it may reasonably be concluded that tearing of the tissue was not the cause of the period of slow fall of O_2 -tension which occurs on subcutaneous injection.

It was also found that the period of slow fall of O_2 -tension was independent of temperature of the gas injected, between the limits of 14° and 40° C. It was not due to infection; great care was taken to inject only sterile gas; this, however, did not appear to be necessary, since unfiltered room air caused no signs of infection. I have shown earlier (5) that pituitary extract and adrenalin caused a fall of O_2 -tension in injected air; and further evidence of this is given later in this paper. The slow

fall of O_2 -tension described above may then be attributed to temporary hyperæmia though the exact cause of this remains to be determined.

A point to be noticed is that the final O_2 -tension in the abdominal cavity was about 10 mm. Hg higher than that under the skin.

From the above experiments it was clear that following the subcutaneous injection of air or N_2 , the CO_2 -tension becomes constant in about an hour, whilst the O_2 -tension does not become constant for 2-3 days. Experiments on alterations in O_2 -tensions should therefore be delayed until the period of constancy is established. Rist and Strohl(8) published hypothetical curves showing the rate of absorption of different gases and the changes in gas-tensions following injections into the thoracic cavity. Their curves were built up mainly by mathematics, based on physical laws; obtaining a few points only on their curves from actual observations, they seem to have missed the period of slow fall of O_2 -tension, although they, as well as others they quote, have demonstrated the existence of the period of constant tension.

Subcutaneous injection of pure O_2 . Pure O_2 was absorbed more rapidly than either air or N_2 ; after the injection of 1000 c.c. of pure O_2 , gas remained under the skin for about seven days only, as compared with 30 to 35 days for air and N_2 . Other observers(6) have found that pure O_2 was absorbed more rapidly than air or N_2 when injected into the thoracic cavity. The changes in gas-tensions following the injection of pure O_2 under the skin were similar to those described for air, the same three periods for O_2 -tension changes being observed. As the O_2 was absorbed and fell in percentage, the percentage of N_2 increased.

Subcutaneous injection of pure CO_2 . Pure CO_2 was absorbed so rapidly that 1 hour after the injection of 1000 c.c. most of it had disappeared; after 24 hours both the CO_2 - and the O_2 -tensions were 42 mm. Hg; after 48 hours the tensions were 42 and 33 respectively. Tobiesen(9) attempted to produce artificial pneumothorax with pure CO_2 and found that the gas was absorbed very rapidly.

Final CO_2 - and O_2 -tensions in different rabbits and cats. As already said, subsequent to the first 2-3 days after injection of air or N_2 , the CO_2 -tension both under the skin and in the abdominal cavity remained at about 50 mm. Hg, whilst the O_2 -tension remained at about 20-30 mm. under the skin and at 30-40 in the abdominal cavity. This constancy of tension has been found after many injections into any one animal made during a period of five months. The exact tension varied however in different individuals. My results are summarised in Table III for subcutaneous injection and in Table IV for abdominal injection; apart from

the presence of the gas tho animals had been in the normal resting condition for at least 24 hours. The total number of observations was

TABLE III. Gas-tensions under skin of resting animals.

Animal	No. of obs.	CO ₂ mm. Hg			O ₂ mm. Hg			Period covered by observations days
		highest	lowest	average	highest	lowest	average	
Rabbit A	16	60	41	49	33	18	25	62
" W	16	54	44	48	33	22	25	48
" V	3	55	46	51	8	8	8	52
25 rabbits	80	69	39	50	37	0	21	—
Cat A	7	46	41	43	28	14	21	32
" B	5	46	40	43	31	12	10	32

TABLE IV. Gas-tensions in abdominal cavity of resting rabbits.

Rabbit W	7	52	43	47	43	38	40	48
" G	5	45	42	43	48	39	42	11
5 rabbits	6	57	44	50	47	29	38	—

115 with rabbits and 12 with cats. As will be seen from Table III, the highest figure for CO₂ obtained with rabbits was 60 mm. Hg, the lowest 39 and the average about 50 mm.; only very few observations varied as much as 10 mm. from the average. These few wide variations were probably due to some unrecognised abnormal condition of the animal at the time the sample was withdrawn. For cats the CO₂-tensions under the skin were appreciably lower, the highest being 46, the lowest 40 and the average 43. The respiratory centre of the cat is probably more sensitive to CO₂ than that of the rabbit. On the other hand, the O₂-tensions for cats and rabbits were more alike, indicating that in the resting conditions of these animals shut up in cages in the laboratory, the circulatory conditions in the subcutaneous region were much the same. The O₂-tensions varied between 8 and 37 mm. Hg. In only two animals, however, was the O₂-tension below 12 mm.; low figures were always obtained in these two animals. Thus in one of them the O₂-tension was 10 mm. Hg on the third day following the injection of gas and 9 mm. on the twenty-fourth day; in this animal the O₂-tension two weeks after the first injection was 11 mm. Hg and five months after the first injection it was 10 mm. Hg. The three observations on Rabbit V (Table III) which all gave O₂-tension 8 mm. Hg, were made on the seventeenth, twenty-fourth and twenty-seventh days following an injection. In another rabbit the O₂-tension was 18 mm. Hg two days after the first injection and still 18 mm. five months after the first injection. The absolute values of O₂-tensions therefore did not depend on the interval elapsed since the first injection. O₂-tensions depended in part upon muscular activity, since muscular exercise increased the O₂-tension by from 5 to 10 mm. Hg sometimes for over 24 hours following the exercise.

The figures for CO_2 -tension in the abdominal cavity resembled very closely those obtained for gas under the skin, the CO_2 -tensions in both situations in normal resting animals being regulated by the respiratory centre. On the other hand, the O_2 -tensions in the abdominal cavity averaged about 40 mm. Hg and were on the whole at least 10 mm. higher than those under the skin, indicating better circulation in the former region. These results agree with those of Henderson and Haggard(3), who obtained 45 mm. in gas in the abdominal cavity of dogs; they, however, did not follow out the tensions beyond a few hours after the injections of the gas. Many observers, *e.g.* Rist and Strohl(8) have obtained about 40 mm. Hg as the normal tension for O_2 in the thoracic cavity.

Cause of the O_2 -tension of the gas under the skin being less than that in the abdominal cavity. Krogh(13) has shown that the circulation in the skin is very free, the capillaries being very patent and easily injected; and that in animals with loose skin the vessels are very tortuous, the skin being well supplied with blood even when stretched. One would therefore expect that the O_2 -tension in the skin itself would be as high as in other regions well supplied with blood, *e.g.* abdominal cavity and thoracic cavity.

From the earlier observations of Pflüger and others it had been deduced that the O_2 -tension in resting muscles was small or nil; this has been confirmed by the recent researches of Verzář(10) and of Krogh(11). Verzář found that the tension in resting muscle was below 19 mm. Hg. In my animals the gas under the skin of the back lay in the tissue spaces between the skin and the muscles, the gas being separated from the capillaries of the skin by the connective tissue of the skin and from the capillaries of the muscles by the connective tissue covering the muscles. Krogh(12) has shown that O_2 diffuses rather more rapidly through muscle tissue than through connective tissue. He has also shown(11) that the capillary circulation in resting muscle is intermittent and concludes that in working muscles, because the circulation is much increased, the O_2 -tension approaches very near to that of the blood. The O_2 -tension in the abdominal cavity was higher than that under the skin, probably because the surrounding muscles being respiratory muscles were contracting rhythmically and were therefore better supplied with blood than the resting muscles under the skin of the back. The O_2 -tension found in the tissue spaces under the skin varied between 8 and 37 mm. Hg; on the whole these limits are, as would be expected, intermediate between those for resting muscle, *viz.* 0-19 mm. Hg, and those in the veins, *viz.*

24–60 mm. Hg. The figures quoted for muscle and for veins were obtained by Verzár from anæsthetised cats. Some results for O_2 -tensions under the skin, both in anæsthetised cats and in anæsthetised rabbits, have been obtained. The O_2 -tension was found to be lowered by 3 mm. to 8 mm. (see Fig. 5) by full doses of urethane; these effects did not alter the limits, 8–37 mm. Hg already given for O_2 -tensions. The CO_2 changes produced by anæsthesia will be dealt with later.

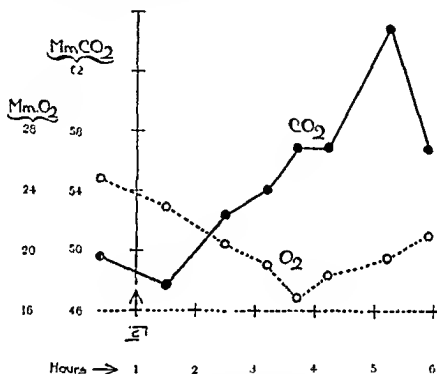


Fig. 5. Subcutaneous injection of a full dose of urethane. CO_2 - and O_2 -tensions in gas under the skin (rabbit).

Effect of vaso-constrictor substances. I have shown in an earlier paper (5) that adrenalin and pituitary increased the CO_2 -tension and decreased the O_2 -tension in air injected subcutaneously. I give here some comparative experiments on the effect of these vaso-constrictor substances on the gas-tensions in air under the skin and in the abdominal cavity. The substances were injected when the O_2 -tension was constant. It will be seen (Table V) that after adrenalin the CO_2 -tension under the skin was increased from 45 mm. Hg to 54 mm. Hg, whilst the O_2 -tension was reduced from 25 mm. Hg to 16; that is, the CO_2 -tension rose to the same degree that the O_2 -tension fell. The tensions in the abdominal cavity were much less altered so that the adrenalin in the amount given caused a greater constriction of the vessels in the subcutaneous region than in the abdominal cavity. Many observers have shown that the action of adrenalin on the vessels of the gut is variable, ordinary doses causing constriction and small doses dilatation.

TABLE V. Rabbit G. Effect of 2 mg. adrenaline chloride subcutaneously.

Time, mins.	Tensions under skin mm. Hg		Tensions in abdominal cavity mm. Hg	
	CO ₂	O ₂	CO ₂	O ₂
0 ...	45	25	45	43
38 adrenalin ...	—	—	—	—
109 ...	51	22	42	43
189 ...	54	16	43	40
219 ...	54	18	40	40
24 hours later	45	29	—	—

TABLE VI. Rabbit G. Effect of 3 c.c. "Infundin" (1 c.c. per kilo) subcutaneously.

0 ...	43	28	43	39
25 "Infundin"	—	—	—	—
77 ...	49	24	49	31
159 ...	53	16	49	30
251 ...	46	17	36	36
366 ...	40	19	—	—
24 hours later	42	29	—	—

Pituitary extract (Burroughs and Wellcome's "Infundin") injected subcutaneously, 1 c.c. per kilo, caused a definite rise of CO₂-tension and a definite fall of O₂-tension both in the abdominal region and in the subcutaneous region (see Table VI). Here again the effects were more marked under the skin than in the abdominal cavity. The CO₂-tension was increased by 15 mm. Hg under the skin and by 6 mm. in the abdominal cavity; the O₂-tension was decreased by 12 mm. Hg under the skin and by 9 mm. in the abdominal cavity. The action passed off more quickly in the abdominal cavity than under the skin. In the doses given pituitary extract had a more powerful action than adrenaline, the results in Tables V and VI being obtained from the same animal.

The effect of pituitary extract was found to vary greatly in different animals; the rise of CO₂-tension under the skin produced by the same dose per kilo may in one animal be double that in another.

It was frequently observed that after the effects of constrictor substances had passed off the O₂-tension in gas under the skin was higher for a time than before the experiment; this was presumably due to dilatation of vessels with increased blood supply. Often also the CO₂-tension was lower after an experiment than before; this must have been due to the respiratory centre in most cases.

The effects of a great fall of blood-pressure upon the gas-tensions under the skin. Histamine was injected subcutaneously in doses sufficient to cause shock. Dale and Laidlaw (14) recommended 50 mg. of the hydrochloride for a cat. Table VII gives details of the effects of subcutaneous injection of 85 mg. ergamine acid phosphate without anaesthesia. The

TABLE VII. Cat A. Effect of 85 mg. ergamine acid phosphate subcutaneously.

Time, mins.	Tensions under skin mm. Hg	
	CO ₂	O ₂
0	46	14
2 ergamine injected	—	—
88	74	6
173	94	3
243	108	2
19 hours later ...	60	10

animal, a cat, showed signs of collapse and shock but could be easily roused and did not appear to sleep. The CO₂-tension was enormously increased from 46 mm. Hg to 108—this is the highest figure for CO₂ under the skin obtained in a living animal. The O₂-tension was markedly decreased from 14 mm. to 2. Histamine causes a profound fall of blood-pressure which practically stops the circulation in some organs; some organs become dark plum-coloured according to Dale and Laidlaw (15). The fall in O₂-tension was evidently due to sluggish circulation. The rise in CO₂-tension was due in part to this but also to another factor; the CO₂ capacity of the blood was markedly reduced by histamine (16), owing to the oxygen "want" in the tissues causing acidosis.

Reference has already been made to the temporary and excessive rise of CO₂-tension under the skin following severe muscular exercise, and due to the formation of lactic acid in the underlying muscles; the highest rise produced by muscular exercise was from 42 mm. to 76 mm. Hg. Anaesthetics also caused a marked increase in CO₂-tension. Fig. 5 illustrates this point; the rise in CO₂-tension may be partly circulatory since the O₂-tension falls slightly, but it is evidently connected also with the formation of acid substances in the tissues, e.g. muscles; the sharp rise on the CO₂ curve in Fig. 5 resembled that produced by muscular exercise, that is, by lactic acid. Ergotoxin produced effects similar to those caused by urethane, the CO₂-tension rising from 47 mm. to 62 mm. Hg after intravenous injection of 7.6 mg. ergotamine tartrate, whilst the O₂-tension fell only from 19 to 14 mm. The marked rise in CO₂-tension was probably due, in part, to formation of acid substances in the tissues, e.g. muscles. The fall in O₂-tension was probably due to circulatory changes; Dale (17) has shown that ergotoxine causes the arterial muscle to contract but paralyzes the vaso-constrictor nerves. There were many conditions in which the CO₂-tension was increased to a much greater extent than the O₂-tension was decreased but this phenomenon was best marked in histamine shock. The opposite condition was sometimes met with after subcutaneous injection of insulin, the

subcutaneous injection of gas may be used in estimating the alveolar CO_2 -tension clinically.

5. Constriction of subcutaneous blood vessels lowers the O_2 -tension under the skin and raises the CO_2 -tension to about the same degree. Both adrenaline and pituitary extract produce greater effects on gas tensions under the skin than in the abdominal cavity.

6. Formation of acid substances, such as rapid production of lactic acid in muscle tissue by severe exercise, greatly increases the CO_2 -tension in the overlying subcutaneous region. Similar marked increase of CO_2 -tension is produced also by histamine shock, by urethane and by intravenous injection of ergotoxin; the O_2 -tension in most of these cases is much less altered, being increased after muscular exercise, but decreased in the other cases.

7. The respiratory centre regulates the CO_2 -tension in gas under the skin of normal resting animals; on the other hand, the circulation in the surrounding muscles and subcutaneous tissue regulates the O_2 -tension in gas under the skin of normal resting animals.

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A METHOD FOR THE GRAPHIC REGISTRATION OF
OXYGEN-CONSUMPTION AND CARBON DIOXIDE
OUTPUT; THE RESPIRATORY EXCHANGE IN
DECEREBRATE RIGIDITY¹. By J. G. DUSSER
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DECEREBRATE rigidity discovered by Sherrington, as far back as 1896, is in many respects an interesting phenomenon. One of us⁽¹⁾ has shown that the contraction of the extensor muscles in this form of "continuous" hyperinnervation⁽²⁾ is accompanied by very distinct action currents which resemble in many respects the action currents of striped muscle in voluntary contraction in man. From this fact the contraction of the muscles in decerebrate rigidity might be looked upon as a tetanus. But in contradiction with this view is the statement by Roaf⁽³⁾ that in decerebrate rigidity there is no increase in the respiratory exchange. On the other hand, Lovatt Evans, according to a private communication to one of us (D. de B.), carrying out experiments in 1911 to ascertain the effect of removing decerebrate rigidity by curari, noticed "at constant temperatures, a fall in the carbon dioxide, but as he was not quite satisfied with the method employed to determine the gaseous metabolism, and as, in the meantime, Roaf's experiments on the subject appeared, he discontinued the experiments."

Bayliss has given some information on the heat production in muscles in decerebrate rigidity. He⁽⁴⁾ refers to these experiments as follows: "Although the work is not yet complete, I found that there is a certain amount of heat produced, in magnitude varying with the degree of tonic contraction, although it is undoubtedly very much less than that produced in an artificial tetanus of a similar height."

Since the experimental evidence did not seem to be conclusive, we thought it desirable to study once more the respiratory exchange in decerebrate rigidity. We made use of a method which not only enabled us to read graphically the oxygen consumption, as in the method of Krogh, but also to record graphically the carbon dioxide output and therewith the respiratory quotient.

¹ A Preliminary Communication was given in *Klin. Wochenschr.* 1924, p. 395.

Method. We used a small spirometer of the pattern of Krogh⁽⁵⁾ of ca. 1.5 litres capacity, *i.e.* the capacity of the lid, large enough to allow experiments on cats for about 10–20 minutes. Through an inspiratory valve (Müller-valve of only 2 mm. water resistance), connected with the tracheal cannula, the animal inspires the O_2 contained in the spirometer and through an expiratory valve it expires back into the same spirometer. But the CO_2 is absorbed by the soda lime on the bottom of the spirometer, so that only the O_2 not used by the animal is given back into the spirometer. By the respirations of the animal and its oxygen consumption a rhythmically moving, gradually falling curve is written on the kymograph and the amount of fall in mm. at the end of an experimental period gives, after calibration of the spirometer, the amount of O_2 consumed during that period.

With this method, however, only the oxygen consumption can be recorded; the CO_2 output must be measured by one of the known procedures, *i.e.* estimation by weighing or one or other chemical analysis (method of Haldane, etc.).

There are already some methods of graphic estimation of the respiratory exchange, the CO_2 -output inclusive. The method of Hanriot and Richet⁽⁶⁾, necessitates the use of three gas meters; it seems to be a reliable method, notwithstanding that so many gas meters are necessary, but a real graphic record of the respiration is not obtained, only a differential record of the velocity of two of the gas meters. The method of d'Arsonval⁽⁷⁾ is to absorb the CO_2 by potassium hydroxide, to set it free again by sulphuric acid and to collect this liberated CO_2 in a spirometer, the rise of which is registered graphically. Obviously this method does not allow for a real graphic record of the respirations of the subject experimented upon. Finally, the method of Fano⁽⁸⁾ must be mentioned; this method, apparently obsolete, has been used by the author for very small animals and gives only a gradually declining line according to the absorption of the CO_2 , but no true record of the respirations. Thus none of the methods which, so far as we know, have been published gives, as does our method, a true graphic record of the rate and depth of the respirations, in short, of the pulmonary ventilation.

To the Krogh apparatus as used for the measuring of the O_2 -consumption we have added what we might call our CO_2 -trap, through which it is possible to record graphically the CO_2 -output and to get directly an impression and very easily an accurate estimation of the r.q. This method of the CO_2 -trap will be best described by referring to the subjoined scheme.

In the upper part of Fig. 1 the Krogh spirometer is seen with the soda lime on the bottom for absorbing the CO_2 given off during the respiration. The capacity of the lid of the spirometer is of about 1.5 litres, which enables, when the spirometer is filled with O_2 from an O_2 -cylinder, to continue the experiment with the animal over a time sufficiently long to

allow of trustworthy results. When the spirometer is used for measuring the O_2 -consumption, the tube leading directly from the expiratory valve to the spirometer (at *a*) is open, whereas the tubes at *b* and *c* are closed.

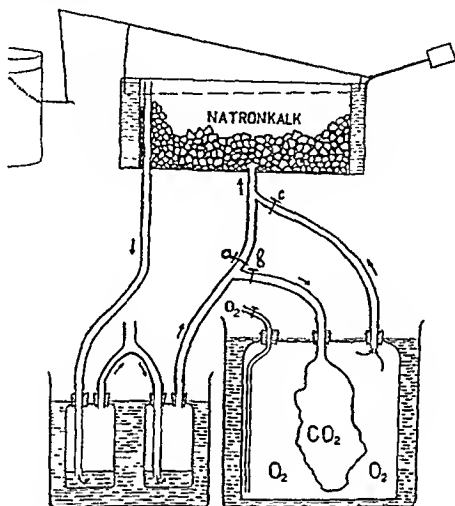


Fig. 1.

When measuring the CO_2 -output, the tube at *a* must be closed and the tubes at *b* and *c* opened. The expiration-air then goes from the expiratory Müller valve along *b* to the CO_2 -trap, i.e. a large rubber balloon, which can take about 14 litres of gas without coming under pressure. This balloon is enclosed airtight in a larger vessel filled with O_2 and put in a water bath or another heat-insulating device to keep the gas at as equal a temperature as possible. When the animal makes an expiration it expels the air into the rubber balloon where the expired air is caught, as in a trap, so that it does not mix with the O_2 of the spirometer, but on the other hand acts volumetrically and, by distending the rubber balloon, expels exactly its volume of O_2 from the larger surrounding vessel, along *c*, back into the spirometer. Thus by this method the CO_2 of the expired air is not re-breathed, whereas, because it is not absorbed, it can act volumetrically as if entering the spirometer. The result is that on using the CO_2 -trap one gets a less sharp falling curve. The difference in fall

between the O_2 -curve and the CO_2 -curve is equivalent to the expired CO_2 over the period of the curve; the difference being that during the writing of the O_2 -curve the CO_2 was absorbed by the soda lime in the spirometer, whereas during the writing of the CO_2 -curve it is not absorbed, only caught in the trap and so can act still volumetrically.

As soon as the rubber balloon is filled, the wall comes under pressure and the CO_2 -period must be broken off, because the animal would else have to breathe against the rapid increasing pressure of the elastic rubber wall. This is directly seen in the curve by a rapid bending down of the respiratory line.

A scheme of the two curves, the O_2 - and the CO_2 -curves, is given in Fig. 2.

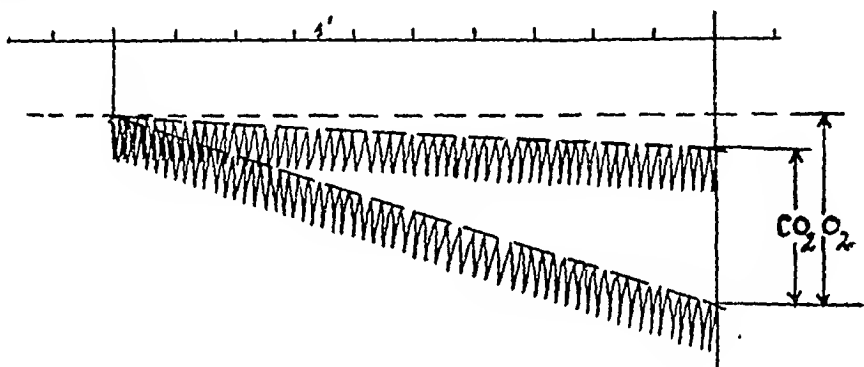


Fig. 2.

From these curves one gets immediately information about the R.Q. When the R.Q. is smaller than 1, the CO_2 -line will fall. When this line is horizontal, it means that the intake of O_2 is exactly the same as the output of the CO_2 , i.e. that the R.Q. is 1, whereas when the CO_2 -curve is rising above the horizontal line, it means that the output of CO_2 is greater than the O_2 -consumption, i.e. that the R.Q. is greater than 1. There is a further advantage of this method. Those methods in which, as one of the factors for the estimation of the respiratory exchange, the volume of the expired air is measured, give the *apparent* R.Q. only, because the volume of the inspired air always differs from that of the expired air when the R.Q. is not unity; a further calculation is then still necessary to obtain the *real* R.Q. In our method the real R.Q. is directly written down.

As rubber is not impermeable to various gases, e.g. to CO_2 , it might be thought that the measurement of the CO_2 with a rubber balloon as a CO_2 -trap is not quite exact. Special estimations, however, with the large Haldane apparatus, have shown that this diffusion does not play a rôle in the short experimental periods of 10–20 minutes, over which our experiments go; estimations of the CO_2 -percentage of the air in the trap immediately after a CO-period and after 20 minutes always gave the same figures.

According to a kind suggestion, made to us by Prof. Zwaardemaker, it is even possible to obtain an exact measurement of the expired CO_2 and also of the consumed O_2 in the course of a single experiment; for this it is necessary to begin the registration with the writing of a CO_2 -curve; at the end of this period the drum of the kymograph is stopped and by one or other method the CO_2 in the trap is absorbed by potassium or sodium hydroxide. The trap will diminish in volume and an exactly corresponding volume of O_2 be sucked back from the Krogh spirometer into the vessel containing the trap. This causes the writing-point of the Krogh spirometer to fall until all the CO_2 in the trap has been absorbed. Because the drum has been stopped, the writing-point of the spirometer, in descending, writes a vertical line and this descent gives the amount of CO_2 expired and at the same time, in the distance from the horizontal, the amount of the O_2 used during the experimental period. The regularity and straight decline of the CO_2 -curve are the proof that the experimental circumstances, during the period concerned, were regular, so that this period may be relied upon. We did not yet make use, however, of this modification because we did not like to introduce KOH in our rubber balloon. We propose to use it in our experiments on man (see p. 23).

To obviate rapid fluctuations of temperature, the vessel containing the CO_2 -trap must be placed in a larger vessel with water or in some other heat-isolating apparatus; it is therefore also desirable to put the Muller valves into a water basin; the temperature of the expired air is thus brought down to room-temperature. The temperature of the system is read by a thermometer placed in the Krogh spirometer and under these precautions remains fairly constant for a long time; of course the temperature in the spirometer is read at the end of each experimental period. It will be obvious that the whole system behind the Muller valves is saturated with water-vapour at room temperature.

After taking a CO_2 -curve the trap is full of expired air and has to be emptied; this is done by blowing O_2 from a cylinder into the vessel containing the trap along the narrow tube shown in Fig. 1. In order to keep the system also in temperature equilibrium during emptying of the trap, it is advisable to let the O_2 , on its way from the cylinder to the flask containing the trap, pass through a narrow brass spiral tube surrounded by a water jacket.

Still, notwithstanding these precautions, it might happen that the temperature of the system undergoes a slight deviation in one or another direction during the experimental period; this would interfere of course with the gradual decline of the respiratory curve. It is, however, very

easy to take these slow variations into account. The only thing required is to let go the drum, with the writing point of the spirometer attached to it, before the beginning and after the end of each CO_2 -period but without the animal breathing into the apparatus. The tube from the Müller valves to the tracheal cannula is therefore clamped and the animal breathes quietly through the side-way of the cannula. Thus the apparatus is closed and when the drum is now started, the writing point of the spirometer will write a horizontal line on the drum, provided that there be no gradual change of temperature inside the apparatus. Any gradual rise or fall of temperature must show itself by a slow rise or fall of the writing point. After a few minutes the animal is connected with the apparatus and the side-way of the tracheal cannula shut, so that the CO_2 -curve is begun. At the end of the CO_2 -period the apparatus is made a closed system again and a second "temperature-control line" written.

To find then the true descent of the CO_2 -curve during the experimental period we have only to measure in the middle of the curve, as shown in Figs. 3 and 4 for two different types of temperature changes. In this way one is quite independent of eventual slow variations in the temperature of the apparatus. Abrupt deviations of temperature do not occur when the heat-isolation has been established as described.

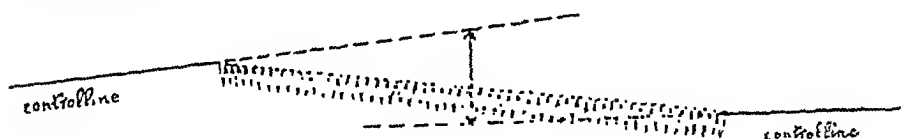


Fig. 3



Fig. 4

In the number of the *Klinische Wochenschrift* in which our preliminary notice appeared, there appeared also a short communication by Helmreich and Wagner on the same problem (viz. the estimation of the CO_2 -output with a Krogh spirometer) in an analogous way to ours. These authors use a rubber bag inside the Krogh spirometer as a CO_2 -trap. We had rejected this method, because the capacity of this spirometer for CO_2 curves in man is too small. The capacity of the Krogh spirometer for O_2 -measuring in man (original specimen from Krogh's laboratory) is somewhat less than 23 litres. Supposing that the authors use a rubber bag filling up nearly the whole of the spirometer at the end of a CO_2 -period, the capacity of their trap may be estimated at 22 litres, though this is, no doubt,

too high, since the lid of the spirometer gradually falls. Assuming the frequency of the human respirations to be 18 per minute and the tidal air to be 0.4 litre, the authors can, with their apparatus, continue a CO_2 period for not longer than $22/18 \times 0.4 = \text{ca. } 3$ minutes, a length of period much too short to give reliable results. As is well known, the shortest period allowed for in an O_2 estimation with the Krogh or Benedict apparatus in man is 10 minutes. The authors state that their experimental results have been satisfactory, we do not doubt that this statement may be true for a period of 2 or 2.5 minutes or of perhaps at most 4 minutes when experimenting on children, but that is, as already said, much too short.

With our method we always discard the first minute or more of the curves, in order to be sure to have in every respect equilibrium conditions. The most important of these is the adaptation of the animal to the resistance of the apparatus. This resistance is in our apparatus for experiments on cats not more than 10 mm. water, as read by the height of the water column in the inspiratory Müller valve during expiration. Although we did not find it necessary, the resistance of the apparatus may be readily diminished by taking some what wider tubes than ours which had 7 mm. internal diameter.

Having found that our method gave satisfactory results in experiments on animals, we proceeded to design an analogous apparatus for respiration experiments in man. Instead of a rubber balloon we made use of a large spirometer of about 130 litres capacity as a CO_2 trap, this large apparatus allows experiments lasting about 15 minutes, to be made on man. We will describe this larger apparatus in a future communication.

The experiments on the respiratory exchange in decerebrate rigidity, in which this method of graphic registration of the O_2 consumption and of the CO_2 -output was first of all used, were made on the cat, decerebrated by the earlier Sherrington method, *i.e.* trephining of the skull and cutting through of the brain stem in front of the tentorium cerebelli with a blunt knife. The vagi were rigorously kept intact while tying the carotid arteries and in consequence the animal breathed spontaneously quite regularly and with normal frequency. After the decerebration, the animal was put in a water bath of body temperature ($38\text{--}39^\circ \text{C}$) and the water level was so adjusted that the animal was standing on the bottom of the bath on its four legs, but the chest and abdomen were only slightly submerged, thus the respiratory movements were not hindered by the pressure of the water. Before beginning an actual respiration experiment we mostly waited about one hour and sometimes longer (up to three hours) to allow the anaesthetic (ether) to disappear from the animal.

First an O_2 -curve was written, followed by a CO_2 curve with use of the trap, and then again an O_2 followed by a CO_2 curve. Then we proceeded to abolish the decerebrate rigidity, either of the four limbs by cutting through the N. ischiadicus, N. femoralis and the brachial plexus on both sides, or of all the extensor muscles by urethane narcosis (ca. 400 mgms. pro kilo intravenously¹), followed after some time by 2 or 3 c.c.

¹ This is the dose found by Storm van Leeuwen and Le Heux as the average

of a 25 p.c. solution of ethyl urethane subcutaneously; this gives a very quiet, long lasting narcosis without any disturbance of the heart or of the respiration. After the abolition of decerebrate rigidity O_2 - and CO_2 -curves were again taken, and from the difference between these and the curves during decerebrate rigidity it was possible to judge of the respiratory exchange in this state of contraction.

The temperature of the animal (per rectum) and of the Krogh spirometer were taken at the end of each experimental period, as well as the frequency of the respiration in the course of such. The volumes of air as measured from the curves are all reduced to dry air of $0^\circ C.$ and 760 mm. Hg. and given per kilo cat and per hour.

From time to time the apparatus was tested to see that it was air-tight by weighting the lid of the spirometer with a weight of 40 gm., which resulted in a small depression of the curve during the time the weight was applied on the lid. As soon as the weight was taken away, the curve took up again its original line of decline, showing that there was no leakage of air from our apparatus.

EXPERIMENTAL RESULTS.

As a paradigm of our experiments we will give here first of all the subjoined protocol of one of them:

Cat, 3.42 kilo. Fasting 48 hours. Barometer, 750 mm. Hg.

Ether anaesthesia; tracheal cannula; both carotids tied; vagi kept intact. Decerebration at 3 p.m. Ether off. Regular spontaneous respirations. Decerebrate rigidity soon well developed. Animal in thermostat.

1st O_2 -curve (curve a). In rigidity	3.37-3.51	Temperature spirometer	12.8°
		Rectal temperature	38.7°
		Frequency of respirations	21 p.m.
2nd O_2 -curve (curve c). In rigidity	4.14-4.25	Temperature spirometer	13.7°
		Rectal temperature	38.7°
		Frequency of respirations	21 p.m.
2nd CO_2 -curve (curve d). In rigidity	4.39-4.50	Temperature spirometer	13.6°
		Rectal temperature	38.7°
		Frequency of respirations	23 p.m.
5.30-5.41. On both sides the N. ischiadicus and femoralis and the brachial plexus are cut, without any bleeding.			
1st O_2 -curve (curve g). Flaccid	5.50-6.04	Temperature spirometer	13.8°
		Rectal temperature	38.7°
		Frequency of respirations	20 p.m.
1st CO_2 -curve (curve h). Flaccid	6.10-6.25	Temperature spirometer	13.7°
		Rectal temperature	38.8°
		Frequency of respirations	19 p.m.
2nd O_2 -curve (curve k). Flaccid	7.59-8.11	Temperature spirometer	11.1°
		Rectal temperature	38.4°
		Frequency of respirations	24 p.m.

intravenous dose out of twelve experiments for extinction of the homolateral flexion reflex in the decerebrate cat. See J. W. le Heux, *Pflüger's Arch.* 174. p. 115. 1919.

From Fig. 5 it can be seen that the O_2 -consumption in the curves *a* and *c* during decerebrate rigidity is distinctly greater than in the curves

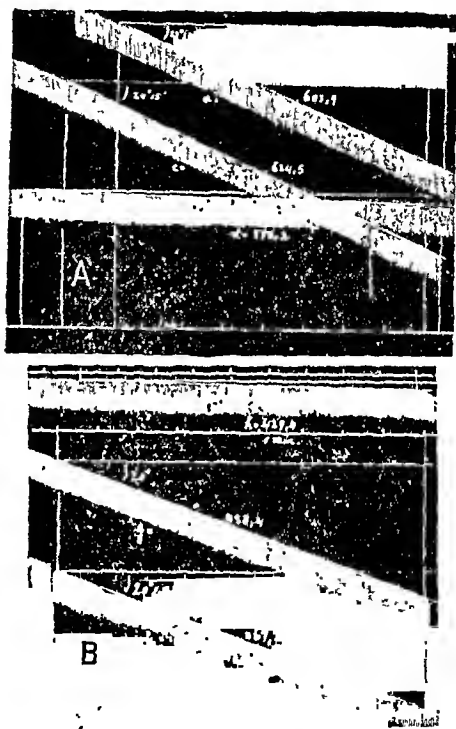


Fig 5 Cat O_2 consumption and CO_2 output
 A—during decerebrate rigidity
 B—when rigidity abolished (Time in minutes)

g and *k* during abolition of decerebrate rigidity in the extensor muscles of the limbs. The CO_2 -output has also diminished, but the *r q*. has practically not changed, as can directly be seen from the fact that the decline of curve *h* is essentially the same as that of curve *d*.

The O_2 -consumption in the different periods was:

In period of curve a : 603.9 c.c. }
 " " c^* : 624.5 c.c. } ; during decerebrate rigidity
 " " g : 458.4 c.c. }
 " " k : 467.3 c.c. } ; during abolition of rigidity

The CO_2 -output in the different periods was:

In period of curve d^* : 576.2 c.c.; during decerebrate rigidity
 " " h : 429.4 c.c.; during abolition of rigidity

* During curves c and d the animal now and then showed twitches in some of its muscles.

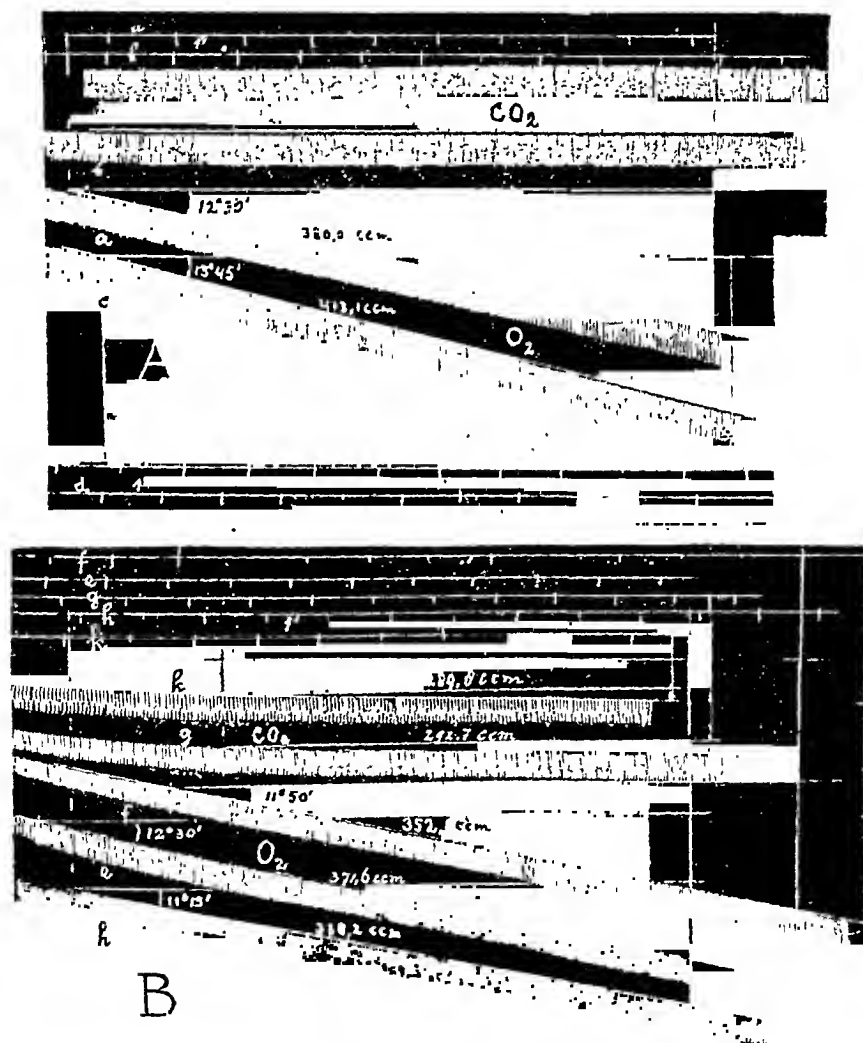


Fig. 6. As Fig. 5.

A set of curves from another cat showed a diminution of the O_2 -intake and CO_2 output after abolition of decerebrate rigidity through ethyl urethane (see Fig 6) Fig 6 A gives the curves during decerebrate rigidity, Fig 6 B those after abolition of the rigidity

Interesting is the fact that in curve *c* the O_2 consumption after urethane is nearly the same as in curve *a* during decerebrate rigidity The explanation is that at the end of curve *c* it was found, that notwithstanding the administration of 45 cc of a 25 p c urethane solution intravenously there was still a very distinct rigidity in all four limbs, strongest in the hind legs Therefore yet 2 cc urethane were injected, after which the animal became flaccid and the subsequent curves were taken

In curve *h* of Fig 7 the effect of the coming under pressure of the rubber balloon, when filled by the expirations of the animal, can be seen At the end of this curve it rather abruptly bends downwards

In Fig 7 we give the results of our five experiments in which the animals were kept in as equal conditions (especially of temperature) as

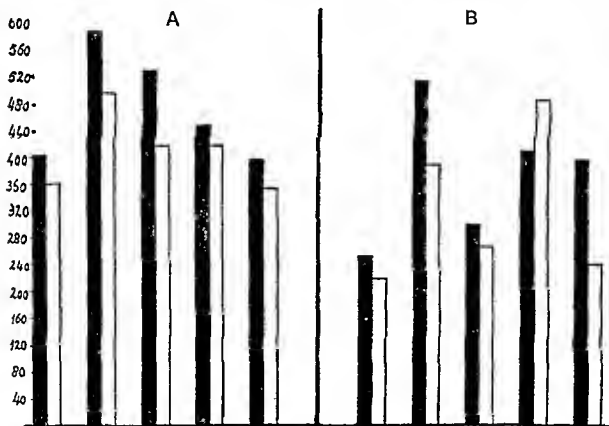


Fig 7 Cat A O_2 consumption B CO_2 output Black columns = during decerebrate rigidity White columns = when rigidity abolished

possible by placing them in a thermostat, the results of our earlier experiments point in the same direction, but because in these the animals were not kept in so rigorous conditions with regard to their body tem-

perature, we do not reproduce them here. The results of the five experiments are, so far as we can see, fairly conclusive. In all the O_2 -consumption was distinctly lower after abolition of the decerebrate rigidity, the largest difference being nearly 23 p.c. (Exp. 3). In one of our earlier experiments, where the animals were not yet kept in a water-bath and where the artificial heating was done by warming the operation table on which the animal was lying, we even have got a difference in the O_2 -consumption, before and after abolition of the rigidity, of 25 p.c. (469, 2 c.c. against 350, 6 c.c.).

With regard to the CO_2 -consumption, it follows from Fig. 7 that in four of the five experiments, there was a distinct diminution of this factor after abolition of the decerebrate rigidity. Only in one experiment was there a rise of the CO_2 -output after cutting of the nerves of the limbs. How this fact must be explained is not easy to say; we think that perhaps the most plausible explanation is that the cutting of the nerves acted as a strong stimulus, which in this particular animal gave rise to an abnormally high outpouring of adrenalin by the suprarenal capsules. This supposition was tested by observing the effect of an intravenous injection of adrenalin on the CO_2 -output by the trap method; the administration of adrenalin was followed by a distinct augmentation of the R.Q. Although this might be looked upon as evidence of the above-mentioned explanation, we do not regard it as conclusive; probably the phenomenon is much more complicated.

So far as our experimental evidence in this matter goes, we might conclude that it shows that decerebrate rigidity is accompanied by a distinct augmentation of the O_2 -consumption and in most cases (four out of five) by an augmentation of the CO_2 -output of the animal. Although this augmentation of the respiratory exchange under decerebrate rigidity is distinct, it is much less than the augmentation of the respiratory exchange during phasic innervations and movements. This is already shown by the augmentation which, as mentioned, was present during the curves *c* and *d* of the experiment of Fig. 5, during which experimental periods clonic twitches occurred in some of the muscles of the animal. More distinctly still this was shown in another experiment in which the animal made small phasic movements with the hind limbs and trunk; during these phasic movements the O_2 -curve showed an immediate increase in the O_2 -consumption.

SUMMARY.

1. A method is described by which it is possible not only to record graphically the oxygen-consumption, as in the methods of Krogh or Benedict, but also the CO_2 -output (method of the CO_2 -trap).

2. With this method it is shown that, so far as our experimental evidence goes, there is a slight increase of the O_2 -consumption and CO_2 -output during decerebrate rigidity. The largest difference that was found in these experiments between the O_2 -consumption during decerebrate rigidity and after abolition of this form of continuous hyperinnervation, was 25 p.c.

3. The respiratory exchange during "phasic" innervations and movements is much greater than during decerebrate rigidity.

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ADDENDUM

It might be argued that perhaps during the writing of an O_2 curve the air in the spirometer will be not quite saturated with water vapour, whereas during the writing of a CO_2 curve the water vapour of the expired air is not absorbed by the soda lime, because caught in the CO_2 trap. This factor might result in a somewhat too high R/Q . This objection, though theoretically correct, in reality falls to the ground for the following reasons:

The difference in the tables for reduction of dry and wet air, both to dry air of 0°C and 760 mm Hg, for temperatures between $12-20^\circ \text{C}$. is from 1.2-2.1 p.c. In reality this difference is in our method much smaller yet, as Roth (*Boston Med. and Surg. Journ.*, April, 1922) has shown, that in a spirometer with water seal and soda lime on the bottom the average degree of moisture is still about 73 p.c. with regard to saturation; we may take it that the difference mentioned above is in our experiments, therefore, to be no more than 0.3-0.6 p.c. When using water valves, as we did, this factor becomes practically negligible because the animal takes in the air above the water seal in the inspiratory valve, which may be regarded as at least nearly, probably entirely, saturated with water vapour. The assumption in our paper, that the whole system, under our conditions, is saturated with water vapour of the temperature read off on the thermometer in the spirometer, i.e. room-temperature, is, as we think, safe.

THE REGULATION OF THE BREATHING AFTER
THE INGESTION OF SUGAR. BY C. G. DOUGLAS
AND J. G. PRIESTLEY.

(From the Physiological Laboratory, Oxford.)

THE principal alterations shown by the respiratory exchange as a result of the consumption of carbohydrate are, of course, well established, but not much attention has been directed to the variations of the breathing in these circumstances, though the alteration in the respiratory exchange which results in a rise of the respiratory quotient, and shows itself mainly in an increase in the CO_2 -output, affords an opportunity for investigating the accuracy with which the breathing is coordinated with small changes in the metabolism of the body.

Tögel, Brezina and Durig⁽¹⁾ have shown that the respiratory quotient rises more quickly and mounts to a higher figure after taking lævulose than it does after taking glucose, and these results have been confirmed and extended by Higgins⁽²⁾. The latter tested the effects of various sugars on the human subject in the "post-absorptive" state, and found that the respiratory quotient began to rise within a few minutes after taking 100 gm. of either cane sugar, lactose or lævulose. The respiratory quotient reached its maximum some 20 to 30 minutes after taking the sugar and then gradually declined. On the other hand when glucose or maltose were taken the respiratory quotient did not begin to change materially till 20 to 30 minutes had elapsed and then it only rose slowly, the gradual rise continuing for a considerable time. The later observations of Bornstein and Holm⁽³⁾ on the effects of glucose and lævulose are in substantial agreement with those of Durig and his colleagues and Higgins. In view of these results it seemed to us that cane sugar would be perfectly satisfactory for our purpose, for we might anticipate that the respiratory quotient would rise both rapidly and considerably, and that the effect of the sugar on the respiratory exchange would pass off in a reasonable length of time.

One of us (C. G. D.) acted throughout as the subject of the experiments. The observations were made in the forenoon, the subject having had no food since dinner the previous evening, *i.e.* 14 to 15 hours before the experiments began. During the whole course of an experiment the

subject reclined in a deck chair and tried to remain as quiet as possible, but as he was not lying in a condition of complete rest, the total respiratory exchange was naturally somewhat greater than would have been the case in a strict experiment on the basal metabolism.

Determinations of the total respiratory exchange were made by the bag method, the volume of air per breath being calculated from the number of breaths given to fill the bag. The composition of the alveolar air was determined by the Haldane-Priestley method, samples taken at the end of inspiration and the end of expiration being received into evacuated gas sampling tubes fixed just in front of the subject so that he could give the necessary deep expirations down the alveolar air pipe with a minimum of personal disturbance. The cane sugar (80 gm. in one experiment, 75 gm. in the others) was dissolved in two cups of tea (circa 350 c.c. liquid) in order that it might be swallowed as quickly as possible.

After 10 minutes' preliminary rest the total respiratory exchange was determined for a period of 6 to 9 minutes. In some of the experiments a second determination of the respiratory exchange was made 6 to 8 minutes later. The sugar was then swallowed and further determinations of the total respiratory exchange were subsequently made at intervals. When the course of events was only followed for about an hour after taking the sugar the first of these determinations was begun 6 to 15 minutes after taking the sugar, and this was followed by three more determinations at intervals of 6 to 12 minutes. In the longer experiments lasting for $2\frac{1}{2}$ hours after ingesting the sugar the respiratory exchange was determined at half-hourly intervals. Immediately after collecting each bag of expired air, blood was in some experiments withdrawn by a syringe from a vein in the arm for the estimation of the blood sugar by Shaffer and Hartmann's method(4), a free circulation of blood being ensured by immersing the hand and forearm in warm water for a minute or two before making the puncture, while in other experiments alveolar air samples were taken.

The results obtained in the different experiments were very much alike and details are therefore given for but three of these. An experiment in which the determinations of the respiratory exchange were accompanied by blood sugar analyses is shown in Table I.

The ingestion of the cane sugar is quickly followed by the usual increase of the blood sugar percentage and rise of the total respiratory exchange and respiratory quotient. It is impossible to say how far the rise of the quotient is attributable to increased metabolism of sugar and

TABLE I.

Time	Respiratory exchange in c.c. per min. at S.T.P.		R.Q.	Breaths per minute	At 37°, moist, and prevailing barometer		Per- centage of sugar in the blood*
	O ₂	CO ₂			Litres ex- pired air per min.	c.c. per breath	
10.12-10.19	252	221	0.88	16.8	8.57	510	.109
10.28-10.34½	247	196	0.79	15.6	7.70	494	—
10.35			75 gm. cane sugar taken				
10.50-10.56	282	257	0.91	15.7	9.55	608	.145
11.7-11.13	289	280	0.97	15.5	10.20	658	.144
11.25-11.31	292	293	1.00	14.5	10.56	729	.124
11.42-11.48½	292	273	0.93	13.3	9.80	736	—

* Blood taken immediately after collecting expired air.

how far to conversion of sugar into fat. The volume of air breathed per minute increases with the rise in the metabolism, the increased ventilation of the lungs being obtained solely by increasing the depth of the respirations while the rate of the breathing is actually somewhat diminished after taking the sugar.

On the hydrogen ion theory of the regulation of the breathing the ventilation of the lungs should vary in proportion to the mass of CO₂ given off so long as the changes in the hydrogen ion concentration of the blood are brought about only by variations in the amount of CO₂ gaining access to the blood. In Table I the total ventilation of the lungs, as measured by the volume of expired air per minute, increases as the CO₂-output rises, but the change in the breathing is proportionally less than in the CO₂-output. It must, however, be remembered that the measurement that counts in this connection should be the actual ventilation of the pulmonary alveoli where the gaseous exchange takes place between blood and air rather than the total ventilation of the lungs. In those experiments in which we determined the composition of the alveolar air the effective alveolar ventilation could be calculated from the rate of the breathing, the average depth of the respirations, and the percentages of CO₂ in the expired and alveolar air by making allowance for the effective dead space. The results of two such experiments are shown in Tables II and III. In the first of these experiments observations were only continued for an hour after taking the sugar, but in the second they were kept up for 2½ hours, by which time the effect of the sugar on the respiratory exchange had passed off and the various measurements had regained the values shown in the original preliminary period.

In all the experiments in which we had the data required for the calculation the effective alveolar ventilation was found to vary in

TABLE II.

Time	Respiratory exchange in c c per min. at S.T.P		R Q	At 37°, moist, and prevailing barometer			Alveolar CO ₂ pressure mm Hg	Alveolar O ₂ pressure mm Hg.	Effective alveolar ventilation. Litres per min *
	O ₂	CO ₂		Breathls per min.	Litres ex- pired air per min	c.c. per breath			
05-10 13	245	202	0.82	13.5	7.42	650	39.4	105.8	4.44
20-10 28	231	181	0.78	13.7	7.04	614	40.9	100.6	3.85
10 31				80 gm. cane sugar taken					
38-10 46	255	227	0.89	13.7	8.02	586	42.0	104.2	4.70
54-11 00	267	258	0.97	14.0	0.46	635	41.3	105.0	5.41
06-11.12	273	270	0.99	14.8	0.65	652	39.6	110.3	5.93
20-11.26	278	271	0.98	16.0	10.10	631	41.0	104.9	5.71

TABLE III.

26-10 34	240	188	0.78	14.1	7.09	503	40.1	101.0	4.06
10 38				75 gm cane sugar taken					
03-11.11	268	265	0.99	14.4	0.48	658	39.4	106.4	5.81
33-11.40	264	245	0.93	15.0	0.28	619	39.0	105.7	5.45
03-12 10	257	230	0.90	14.5	8.61	594	38.8	107.0	5.15
34-12.42	235	194	0.83	13.9	7.55	543	38.0	107.8	4.43
03- 1.12	240	190	0.79	13.7	7.39	530	41.3	08.0	4.02

* The alveolar air samples were taken immediately after the times given in col. 1.

practically direct proportion to the CO₂-output. The total ventilation of the lungs sometimes showed a similar close relation to the CO₂-output, but as a rule there was a tendency for the variations of the total ventilation to be less proportionally than those of the CO₂-output. This difference was sometimes apparent in only one or perhaps two of the individual determinations in an experiment, but it might also be evident in all the determinations after taking the sugar. This will be more readily appreciated by reference to Table IV where the relevant data obtained in the experiments shown in Tables II and III are expressed in comparative

TABLE IV.

	Comparative values		
	CO ₂ output per minute	Total ventilation of the lungs per minute	Effective alveolar ventilation per minute
<i>From Table II.</i> Preliminary period	112	105	115
	100	100	100
After ingesting sugar	125	114	122
	143	134	141
	149	137	154
	150	143	148
<i>From Table III.</i> Preliminary period	100	100	100
After ingesting sugar	141	134	143
	130	131	134
	122	121	127
	103	106	109
	101	104	99

figures, the lowest values for the CO_2 -output, total ventilation and alveolar ventilation in the preliminary period being set at 100 in each instance.

This table shows in striking fashion how closely the effective alveolar ventilation is correlated with the metabolism of the body, in spite of the relatively trifling alterations of the CO_2 -production involved in our experiments. Tögel, Brezina and Durig's experiments show a general correspondence between the CO_2 -output and the total ventilation of the lungs, but here again the variations of the two values are not directly proportional, the alteration in the total ventilation being sometimes relatively greater and sometimes relatively less than the alteration of the CO_2 -output. Unfortunately there are no data in their experiments by which the effective alveolar ventilation can be calculated.

One would anticipate that in the absence of other disturbing factors the increased ventilation of the lungs would be associated with an increased alveolar CO_2 -pressure. The maximum increase in the total ventilation of the lungs in any of the observations made in these experiments was only 3 litres per minute. Campbell, Douglas and Hobson(5) found that when hyperpnœa was caused by breathing air containing CO_2 an increase of 10 litres per minute in the total ventilation of the lungs was caused by a rise in the alveolar CO_2 -pressure of 2 mm. An increase of 3 litres in the total ventilation of the lungs should, therefore, be caused by a rise in the alveolar CO_2 -pressure of the order of .6 mm. It would hardly be possible to determine with certainty so small a change in the composition of the alveolar air when the analysis had to be limited to but a single pair of samples in each instance, the one taken at the end of inspiration and the other at the end of expiration. Owing to the fact that the metabolism was gradually changing throughout the experiments we felt that it would be no use taking a number of alveolar samples with a view to averaging the results, for this would have occupied so much time that the result could not fairly have been employed with the preceding or succeeding determination of the respiratory exchange for the purpose of calculating the alveolar ventilation.

In point of fact the experiment of which details are given in Table II was the only one in which there was perhaps an indication of a rise in the alveolar CO_2 -pressure after taking the sugar. In the other experiments there was, if anything, a very trifling fall with, however, recovery of the original CO_2 -pressure at a later stage, and this recovery occurred in one case at the end of the first hour after taking the sugar when the metabolism and hyperpnœa were still practically maximal. The most distinct

change of this type occurred in the experiment shown in Table III. This tendency of the alveolar CO_2 -pressure to show a slight though apparently temporary fall made us suspicious that it might be attributable to the same cause as the changes in alveolar CO_2 -pressure that may occur after a meal to which Dodds and Bennett have drawn attention(6). Thus a small secretion of alkaline pancreatic juice would be quite sufficient to account for the slight alteration in the alveolar CO_2 -pressure shown in our experiments, and the hydrogen ion concentration of the arterial blood might actually have been a little higher than it was during the preliminary period in spite of the slight fall in the alveolar CO_2 -pressure. We are the more inclined to regard this explanation as the probable one since the subject of our experiments has been found during the last few years to show quite marked changes of alveolar CO_2 -pressure after ordinary meals. Moreover, the increased breathing in our experiments was obtained mainly, if not entirely, by increase in the depth of the breathing with little or no alteration in the rate: in fact in only one experiment, viz. that shown in Table II, did the rate increase, and then but slightly. The hyperpnea resulting from the experimental increase of the alveolar CO_2 -pressure, or the simple increase of hydrogen ion concentration in the blood, always involves increase of the depth of the breathing more than of the rate.

Incidentally the figures given in Table III show the increased alveolar oxygen pressure which accompanies a rise of the respiratory quotient when the alveolar CO_2 -pressure remains nearly steady. If the respiratory quotient had remained unchanged at .78 the fall of alveolar CO_2 -pressure from the original value of 40.1 mm. to that of 38 mm. shown in the fifth observation would have implied a rise of alveolar oxygen pressure from 101 mm. to 103.5 mm. Actually the alveolar oxygen pressure was 107.8 mm.

We are greatly indebted to H. N. Bradhrooke, T. M. Ling, I. M. Titcomb and W. R. Wood for help in these experiments.

CONCLUSIONS.

The rise of the respiratory exchange and respiratory quotient which results from the ingestion of cane sugar is accompanied by an increase in the breathing. The effective alveolar ventilation, and not the total ventilation of the lungs, was found to vary under these circumstances in direct proportion to the CO_2 -output, and a good example is thus afforded of the delicacy with which the breathing is correlated with relatively small variations in the CO_2 -output of the body.

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FACTORS AFFECTING THE THEORETICAL MAXIMUM WORK OF MUSCLE. BY TOSHIKAZU MASHIMO.

(From the Department of Physiology, University College, London.)

A. V. HILL⁽¹⁾ has proposed that the "theoretical maximum work" of the excited muscle should be calculated from the expression μTl , where T is the maximum tension developed in the isometric contraction, at the natural length l , and μ is a certain constant. The experimental value of μ appeared to be characteristic for each muscle; for instance, $1/\mu$ for sartorius, gracilis or semimembranosus, was 6.9 (according to A. V. Hill⁽²⁾), for gastrocnemius, however, 14 to 17 (according to Meyerhof⁽³⁾). In this paper the constant μ has been further investigated.

Method. The tension lever employed was made from a piece of watch spring, and a light bamboo lever; its vibration-period was about .05 sec. It was calibrated by known weights, a table being made to transform readings of the lever into gms. wt. A muscle was hung, by means of a thin iron wire, to the lever, and the other end of the muscle was supported tightly by the muscle clamp. The iron wire and the muscle clamp served as stimulating electrodes. This clamp was moved by a screw, by means of which the length of the muscle could be accurately adjusted. The arm ratio of the tension lever was 48:1, and since the excursion of the lever was always less than 45 mm. the response of the muscle was small enough to be called practically isometric. The muscle was immersed, except at the moment when it was to be stimulated, in Ringer's solution through which air was bubbled continuously. All the experiments were made during the winter with frogs' muscle.

The difference in the value of μ in the gastrocnemius and sartorius muscles. It was suggested to me by Prof. A. V. Hill that the difference observed between the values of μ for sartorius and gastrocnemius muscles respectively might be due to the fact that the fibres of the gastrocnemius do not run the whole length of the muscle.

To obtain parallel fibres from the gastrocnemius, a small portion about 15 mm. long and 1.5 mm. thick was cut off; the fibres were parallel, but, strictly speaking, the ends of the fibres did not lie perpendicular to the direction of the fibres but somewhat obliquely. The stimulus em-

ployed was a short tetanus of less than .6 sec. duration. For *l*, A. V. Hill used the "natural" resting length; in the present experiments, however,

TABLE I. Strip of gastrocnemius.

Exp.	Highest value of max. tension, i.e. T (gm.)	Length of muscle, giving max. tension T (mm.)	$1/\mu$
1	20.9	13.7	3.9
2	35.5	15.0	4.0
3	54.0	13.7	4.5
4	40.0	15.2	5.0
5	83.5	18.5	5.1
6	31.0	12.0	5.2
7	9.3	11.1	5.3
8	30.0	15.2	6.0
9	49.0	10.9	6.2
			Mean 5.0

TABLE II. Strip of sartorius.

1	94.0	29.3	3.7
2	86.0	29.3	3.7
3	47.0	30.0	3.9
4	29.0	37.0	4.6
5	18.0	33.5	5.7
			Mean 4.04

the natural length of the portion employed could not be measured: hence that length was taken at which the tension developed was maximal.

For comparison with this experiment, and to make both experiments similar, a small portion of sartorius was used, about one-third the width of the complete muscle.

We see that for parallel-fibred strips of sartorius and gastrocnemius muscles the value of μ is approximately the same, confirming Hill's explanation of the difference in the complete muscles. The reason why Meyerhof found so widely different a value of μ is that the quantity l which he used in the expression μTl was the length of the muscle, and not that of its constituent fibres.

Time effect on the value of $1/\mu$. The condition of the muscle gradually deteriorates as it is kept in Ringer's solution, and the value of μ somewhat diminishes. The whole sartorius was employed with a short tetanising stimulus. The results of an experiment are given in Fig. 1 and the accompanying description.

Temperature. There is no considerable effect of temperature on the value of μ . The experiment was performed many times, and there appears to be only a small and unimportant decrease in the value of $1/\mu$ when the temperature is raised.

TABLE III. Typical results obtained from the same muscle, with tetanising currents.

Exp.	Highest value of max. tension, i.e. T (gm.)	Length of muscle, giving max. tension T (mm.)	Temp. ° C.	$1/\mu$
1	44.4	31.0	18	3.4
2	31.5	28.0	7	4.4
3	28.3	28.0	5	3.8
4	38.8	31.0	20	3.2

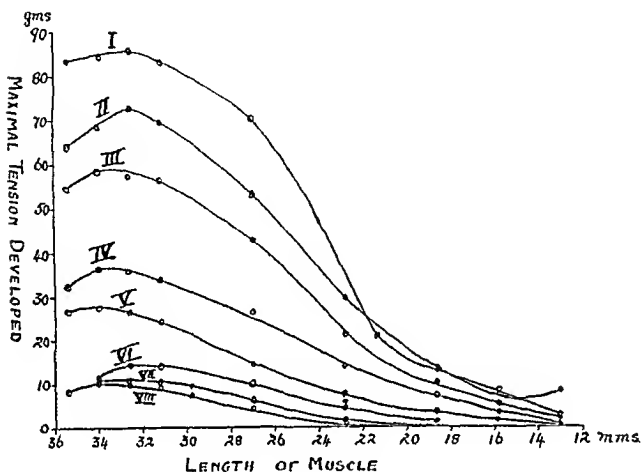


Fig. 1. Curvo

	I	II	III	IV	V	VI	VII	VIII
Time from beginning of exp. in hours	0	1½	3½	4½	6½	23½	27½	29½
$1/\mu$	3.5	3.5	3.2	3.2	3.7	3.9	4.4

Duration of stimulus. A muscle was stimulated with various known durations of the same tetanising current, using Keith Lucas' rotating drum contact-breaker to adjust the duration. There is always a large fatigue effect in such experiments as this, but we have shown above (cp. Fig. 1) that fatigue produces a change in $1/\mu$ in the opposite direction.

TABLE IV.

Duration of stimulus (sec)	Highest value of max. tension, i.e. T (gm)	Length of muscle, giving max. tension T (mm)	$1/\mu$
.05	69.6	22.5	6.5
.05	78.8	22.5	6.2
.2	74.4	22.5	4.7
.5	37.0	22.5	3.8

The alteration shown in Table IV is, therefore, a genuine effect of duration of stimulus.

DISCUSSION AND SUMMARY.

The area of the "tension-length diagram" represents the theoretical maximum work and the ratio of the area to Tl (a quantity of the same dimension as work) is expressed by the number μ . This μ is purely empirical, but it is important in an attempt to estimate the theoretical maximum work without allowing the muscle actually to shorten.

The value of μ was found by A. V. Hill and by Meyerhof to be different in the gastrocnemius and in the sartorius muscles. The writer, however, has found that a portion of gastrocnemius with almost parallel fibres, gives values fairly close to those obtained from sartorius. A. V. Hill suggested that the difference in values obtained from the two muscles is not due to any intrinsic difference in the muscle fibres themselves, but rather to the fact that in the gastrocnemius the fibres do not run the whole length of the muscle: this suggestion is confirmed.

When the experiments are repeated at intervals, the maximal tension becomes distinctly smaller, but μ remains approximately constant. In very prolonged experiments, *i.e.* in experiments lasting a day or more, the value of μ tends to decrease. Temperature has no distinct influence on the value of μ .

Almost all the results described above were obtained with a tetanising stimulus of less than .6 sec.

When the muscle is stretched, the maximal tension is attained rapidly, while if it is allowed to shorten a certain distance, it takes longer than 0.6 sec. for the maximal tension to be developed. But the muscle becomes fatigued very rapidly if excited for longer than this period. Hence the theoretical maximum and the value of μ are affected by the duration of the stimulus.

The writer's thanks are due to Prof. A. V. Hill and also to Dr R. Azuma for their advice and assistance.

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A VAGO-PRESSOR REFLEX. By R. J. S. McDOWALL.

(From the Department of Physiology, King's College, London.)

IN 1915 Bainbridge(1) described a nervous relation between the venous pressure and the pulse rate. He showed that a rise in venous pressure caused a diminution of vagal and an increase in accelerator tone, and pointed out the importance of this factor in the regulation of the cardiac output during muscular exercise. In the course of experiments made for another purpose the possibility arose as to whether the vagus was not also an important factor in the maintenance of blood-pressure under conditions of poor inflow. I am indebted to Dr G. V. Anrep for the information that Pavlov in 1879 had observed that the alterations in arterial pressure which occur as the result of bleeding or of the injection of defibrinated blood were both increased if the vagi were cut. For example, he noted that in dogs the withdrawal of blood to the extent of 1.5 p.c. of their body weight only caused a small or temporary fall of blood-pressure when the vagi were intact but that there was a large fall if the vagi were cut.

It is well known that in hæmorrhage there is constriction of arterioles and this is usually alleged to be due to increased action of the vaso-motor centre. There is also a fall of venous pressure but no one appears to have suggested that this fall is responsible for keeping up of the vaso-motor tone. A series of experiments have been carried out to investigate this point. In all experiments cats anaesthetised with chloralose, and under artificial respiration, were used. Various methods have been adopted to reduce venous pressure.

Alcohol. I have shown elsewhere that a certain amount of alcohol causes a profound fall of venous pressure, although the arterial pressure may be unchanged. The latter observation is well known. If after the administration of alcohol (say 5 to 10 c.c. of a 50 p.c. solution) the vagi be cut there is a marked fall in arterial pressure. This fall may be permanent or may to some extent be recovered from for reasons which will be put forward below.

Histamine. In partial shock caused by the injection of one or two milligrams of histamine there is a fall of venous pressure(3), and inadequate supply of the heart(4). If sufficient histamine is given to reduce

the arterial pressure to 60 or 80 mm. Hg then the cutting of the vagi causes a further fall in arterial pressure.

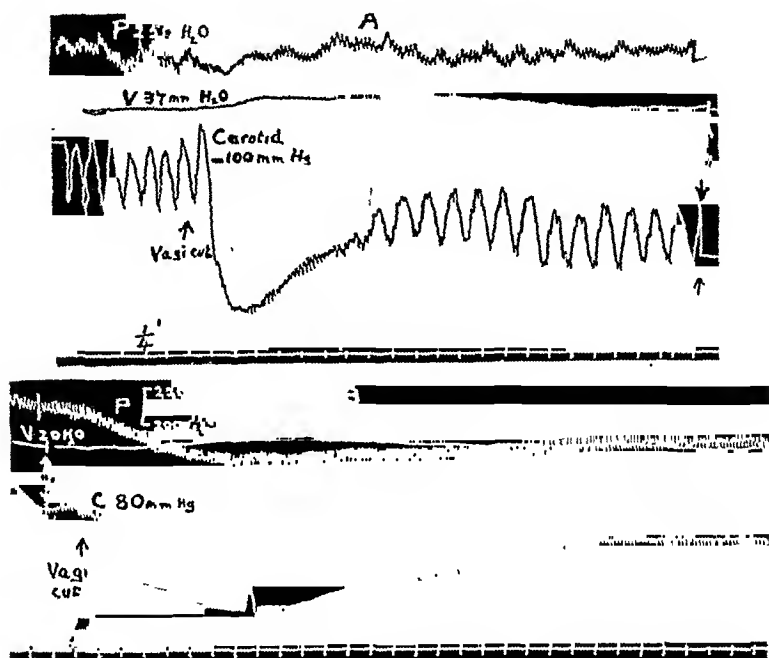


Fig. 1. Effect of alcohol. 8 c.c. of 50 p.c. alcohol had been injected. Tracings of pressure, in pulmonary artery, superior vena cava and carotid artery. Section of the vagi causes a large fall of arterial pressure without a corresponding large rise of venous pressure. In A there is no change of pulmonary pressure. In B there is a fall.

Hæmorrhage. If blood is withdrawn from the animal to lower the arterial pressure to 60 or 80 mm. Hg and, as stated above, also the venous pressure, section of the vagi causes a further fall of arterial pressure (Fig. 2).

Mechanical. A loop of thin twine was placed round the thoracic vena cava and attached to a screw arrangement by which the tension on the loop could be increased and the vena cava compressed. In this way the venous pressure close to the heart could be lowered to any required extent. When the vena cava was sufficiently compressed the arterial pressure fell to 60 mm. Hg, but rose slightly as the compression was maintained. On section of the vagi the arterial pressure at once fell (Fig. 3).

It will be seen from these experiments that under a variety of conditions section of the vagi causes a fall of arterial pressure, whereas normally such section causes, as is well known, a rise in pressure. From

the alcohol experiments it is seen that the effect of the vagal section is not necessarily dependant on a previous fall of arterial pressure. From

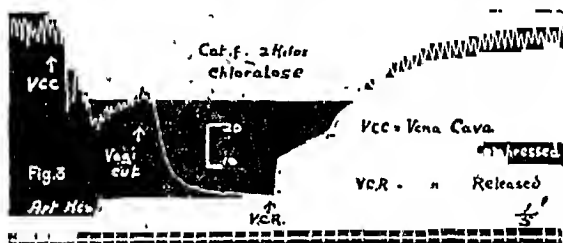
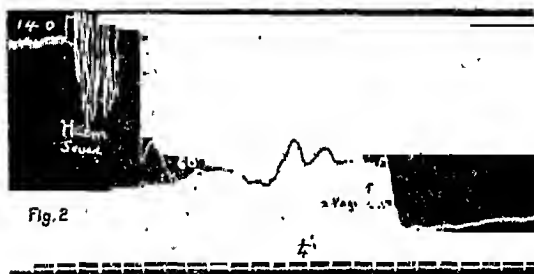


Fig. 2. Effect of hæmorrhage. After a reduction of blood-pressure from 140 mm. Hg to 60 mm. by hæmorrhage, section of the vagi causes a further fall.

Fig. 3. Effect of lowering the venous pressure near the heart. Compression of inf. vena cava at V.C.C. On section of the vagi the pressure falls nearly to zero. V.C.R., vena cava released.

the histamino experiments it is seen that the effect is not due to any specific action of the alcohol, while the hæmorrhage and mechanical experiments exclude any effect of capillary dilation or a fall of peripheral venous pressure. All the experiments have in common a fall in pressure close to the heart. How then can a fall of venous pressure influence the result of vagal section on arterial pressure?

It cannot be considered that the depressor nerve, or what corresponds to it in the cat, is concerned. In those experiments in which the aortic pressure was reduced the diminution would tend to increase the tone of the vaso-motor centre, but section of the vagi in the cat would still further cause an increase in vaso-motor tone.

That the nerve section itself was not responsible for the results was shown by the fact that a similar fall occurred when the vagus paths were interrupted by the application of cocaine to the nerve trunk in the neck.

There appear to be only two possibilities. (1) The increased rate of the heart consequent on the section of the vagi may result in a diminished output when the venous pressure is low. (2) The vaso-motor centre may be under pressor influences which are removed when the vagi are cut.

Changes in cardiac rate. When the venous pressure is low an increased rate of the heart might cause diminished output as a result of diminished filling and this possibility has received special attention. Records of the rate were made by making the heart activate a system of two tambours by means of a hook attached to the anterior surface of the organ. Changes in rate were brought about by the application of hot and cold fluids to the region of the pacemaker. As the peripheral resistance is not affected and may be presumed constant, the blood-pressure may be considered to be an indication of the output of the heart. In this way it is comparatively easy to demonstrate, as pointed out by Henderson, that when the venous pressure is normal or high, an increased rate of the heart increases the output since the organ is still completely filled during the shortened diastole. When the venous pressure is low the rate of the heart within physiological limits makes no difference to the blood-pressure, as would be inferred from Starling's *Law of the Heart*. Of course, if the cooling is excessive, say with iced saline, and the heart rate enormously reduced, the blood-pressure may fall whatever the venous pressure, but in no instance has it been found possible with

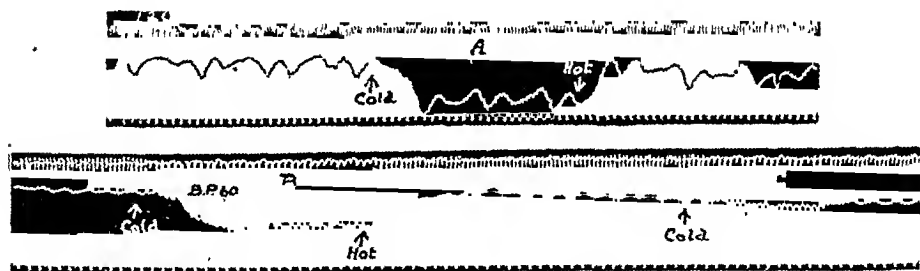


Fig. 4. Effect of varying the rate of heart beat with normal and with low venous pressure. Upper tracing, heart rate. Lower tracing, carotid pressure. Time $\frac{1}{5}$ secs. A. Venous pressure presumably normal since arterial pressure normal; slight slowing of the heart causes a marked fall of blood-pressure. B and C. Venous pressure low after hæmorrhage; in C greater slowing of the heart than in A causes a mere trace of fall; the absence of fall is not due to mechanical conditions since great slowing of the heart produced at B causes a moderate fall.

reflex. For complete confirmation of this and the participation of the peripheral arteries it may be shown that on section of the vagi there is

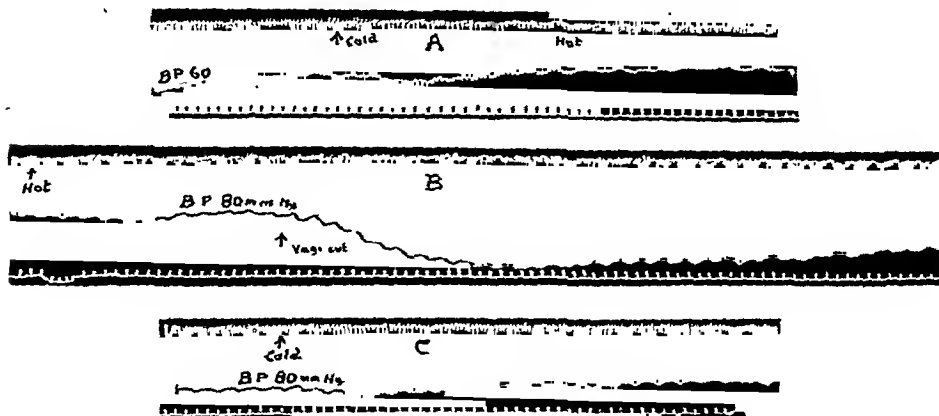


Fig. 6. Upper tracing heart rate, lower tracing carotid blood-pressure after the blood-pressures have been reduced by hæmorrhage. In (B) the typical effect of vagus section is seen. A and C, taken in the same animal immediately before and after the section, show that much larger changes in rate can occur without affecting the arterial pressure. Were the change in the vagal section (B) due to slowing of the heart, a similar change would be expected on slowing in (A), while if the fall in (B) were due to quickening, a recovery would be expected on slowing in (C). Time $\frac{1}{10}$ sec.

in plethysmographic experiments a distinct increase in the volume of a limb and, as is seen in Fig. 7, the increased volume of the limb is main-

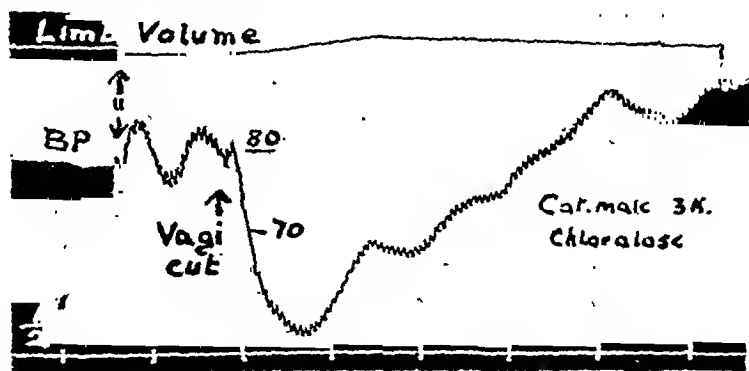


Fig. 7. After hæmorrhage. Section of the vagi causes a fall in arterial pressure accompanied by an increase in the volume of the hind limb.

tained although the arterial pressure may return to normal for reasons stated above. It can therefore be considered that under conditions of low venous pressure in the region of the right auricle, impulses pass up the vagi to the vaso-motor centre which is thereby stimulated and the tone of the arterioles and arteries is increased. An attempt has been

intact vagi so to increase the rate of the heart as to diminish the diastolic filling and the output. The effect of changes in rate are seen in Fig. 4.

To return to the effect of section of the vagus, were the fall of arterial pressure due to the increased rate and a diminished output of the heart it would be possible by again slowing the heart to recover the pressure by peripheral stimulation of the cut vagus (suggested to me by Dr Hewitt) by pilocarpine or direct applications to the pacemaker as above. This has not been found to occur, a fact which is in accordance with the results in relation to the heart rate stated above. When the venous pressure is low it is possible to slow the heart appreciably without affecting the blood-pressure as the increased diastolic filling increases the output per beat and makes up for the diminution in rate (Fig. 5).



Fig. 5. Carotid blood-pressure after hæmorrhage. From A to B, peripheral end of vagus stimulated; slowing of the heart but no fall of blood-pressure. Time $\frac{1}{2}$ sec.

Were the effect of the section due to diminished output it would certainly give the characteristic abrupt rise of venous pressure. This does not occur. Rather there is the equally characteristic slow rise (since smooth and not cardiac muscle is concerned) which I find to be typical of diminished resistance in the arterioles. Fig. 1 also shows that the pressure in the pulmonary artery is not necessarily affected as it certainly would be if the diastolic filling were reduced. In this experiment there was an increased venous inflow to the heart which counterbalanced any fall of pulmonary pressure which may have occurred from pulmonary vaso-dilatation.

The effect of the rate of the heart may further be excluded by increasing the rate by hot applications to the pacemaker prior to the section of the vagi. Then there is the same fall of arterial pressure while the change in the heart rate is negligible (Fig. 6).

The second possibility that the vagi carry pressor influences remains for consideration. That the vaso-motor centre is concerned is shown by the fact that if the centre is cut off by section of the spinal cord in the upper dorsal region, section of the vagi under conditions of low venous inflow does not cause a further fall of arterial pressure. Tone of the peripheral arteries is then, as would be expected, an essential part of the

corpuscles possess very different resistances towards the hæmolyzing agents, we should expect to find in any given preparation of reversibly hæmolyzed blood that some corpuscles have undergone stromatolysis and are completely broken up, that some are much swollen, and that others are only slightly affected; these latter may, under appropriate conditions, appear colourless against the more coloured background furnished by the others.

(b) When reversal is brought about by irrigating a preparation on a microscope slide with saline, the apparent colourlessness of the background might be due to the washing away and dilution of the hæmoglobin solution. Moreover, on no occasion have I been able to get a complete reversion (*i.e.* a preparation in which there was no hæmoglobin in the suspending fluid) except when the hæmolytic agent was made so weak that the preparation was still opaque and that corpuscles were distinctly seen under the microscope; in this case there is no evidence that any corpuscles have discharged their hæmoglobin.

The observations on the refractive indices of the laked and reversed bloods are not only at variance with the rest but are of no value, since the determinations on the reversed blood give, presumably, the refractive index of the serum, or other continuous phase, while those on the hæmolyzed blood before reversion give that of the whole blood, the swollen corpuscles not being separable from the serum by centrifuging owing to their diminished density.

A preferable explanation of the reversal phenomenon lies in the swelling of the corpuscles which would be produced by their suspension in a hypotonic solution; if this swelling were considerable the corpuscular contents would have nearly the same refractive index as that of the surrounding fluid, and the corpuscles would be almost in contact with one another, so that they might very well be invisible both microscopically and macroscopically. In this case, of course, there would be no hæmolysis at all.

Careful observations were accordingly made on the microscopical appearance of blood made reversible by the addition of water and of linolenic acid. Saponin was tried but no reversible preparations could be made.

In the case of water the technique employed was to centrifuge off the corpuscles and to replace a known volume of the serum by the same volume of water. If V_0 is the total quantity of blood taken, V_s the volume of serum replaced by water, and α the corpuscular volume (as determined by the hæmatocrite) then the proportion of serum replaced, β , is given by $\frac{V_s}{(1-\alpha)V_0}$.

β is thus a measure of the hypotonicity of the fluid in which the corpuscles are suspended.

The appearance of a preparation of corpuscles in a hypotonic solution under the microscope depends upon the concentration of the suspending fluid; if this is only slightly hypotonic ($\beta < .45$), the preparation is not completely transparent when viewed in bulk and under the microscope many corpuscles can be seen, most of which show no signs of bi-concavity. A greater degree of hypotonicity leads to the conditions shown in Fig. 1, in which a few fairly normal corpuscles are seen against a background of very indistinct ones. As we increase the hypotonicity further we get the appearance shown in Fig. 2, in which the background has become homogeneous with a number of indistinct corpuscles floating in it. These indistinct corpuscles can be seen more easily after the addition of the dye dianol brilliant blue 6B, in fairly high concentration (Fig. 3). This dye has a large molecule and cannot pass through the corpuscular membrane in its normal state; nevertheless, by increasing the hypotonicity still further ($\beta > .8$) we can get a preparation which is still reversible but which is completely homogeneous

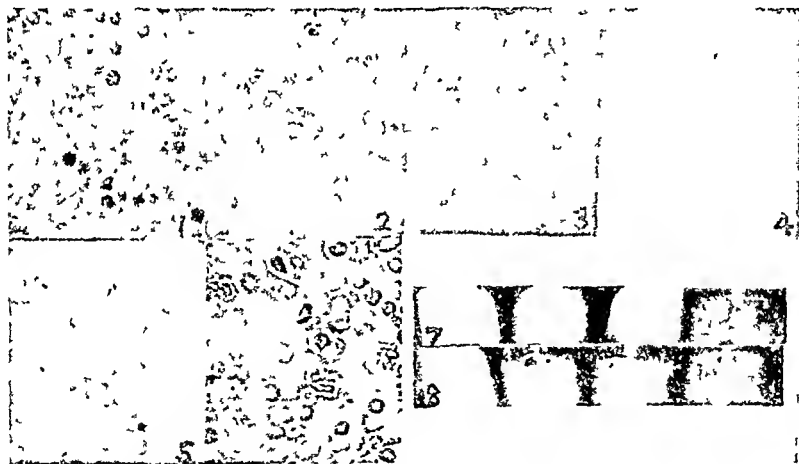


Fig. 1. Water, $\beta = .47$. Magnification 200 diams.

Fig. 2. Water, $\beta = .75$. Magnification 350 diams.

Fig. 3. Water, $\beta = .57$ after addition of dianol brilliant blue

Fig. 4. Linolenic acid .37 %.

Fig. 5. Linolenic acid .32 % after addition of dianol brilliant blue

Fig. 6. Water, $\beta = .75$ after addition of saturated KCl.

Fig. 7. Left to right No hæmolysis, 50 % hæmolysis, laked by linolenic acid, .30 %, 100 % hæmolysis.

Fig. 8. Left to right. No hæmolysis, 50 % hæmolysis, water laked, $\beta = .6$, 100 % hæmolysis.

under the microscope, with or without the addition of the dye. It is suggested that the increasing difficulty in making out the corpuscles is due to a progressive swelling, but this will not be apparent under the microscope since, as mentioned before, the corpuscles can swell without change in diameter.

In the case of linolenic acid, the technique was to add the appropriate quantity of the acid from a micro pipette into a known volume of blood and to shake the whole continually for an hour or so, after this preliminary shaking the linolenic acid seemed to have been dissolved or adsorbed on the corpuscles, as the preparation could be allowed to stand until hæmolysis was complete without risk of the acid separating out as a scum on the surface of the blood.

The hæmolytic action of this acid is somewhat complicated. In concentrations of less than .3 p.c. there is no action and of more than .4 p.c. the action is rapid, the corpuscles becoming spherical and smaller in diameter and then disappearing completely; there are no signs of stromata and the hæmolysis is irreversible. In intermediate concentrations the hæmolysis takes up to 24 hours (at room temperature) for completion, is reversible and gives preparations similar to those obtained by the action of hypotonic solutions (Figs. 4 and 5). The action of high concentrations is thus analogous to that of saponin, as given by Ponder(2) which is one of stromatolysis, the changes in surface tension preceding the solution of the surface membrane causing the corpuscles to take up a spherical form.

It is to be noted that the reversion phenomenon is transient, and the larger the β value or linolenic acid concentration the more rapid is the re-hæmolysis. Hence the corpuscles which are brought down in the reversion by the salt must be permeable to salts. Fig. 6 shows a preparation of water-laked blood to which a small quantity of saturated KCl had been added and which had been allowed to stand for some time. It will be seen that some corpuscles are crenated while others are largely swollen (the solution was considerably hypertonic). Those that are crenated are, presumably, the more resistant ones, and by increasing the hypotonicity during hæmolysis they can be made to disappear. The final picture, then, is the same as that of the solution before any salt had been added. Figs. 3 and 5 are, incidentally, photomicrographs of such re-hæmolysed blood, since the addition of the dye causes reversion (the dye being a tetra sodium salt).

The explanation of the action of the moderate concentrations of linolenic acid is clear; it is a progressive increase in the permeability of the surface membrane of the corpuscle with increasing concentration.

We must suppose that the concentration of hæmoglobin within the corpuscle is such as to give the solution an osmotic pressure which is slightly greater than that of the external medium. This is normally counterbalanced by a difference in salt concentration¹, so that as soon as the membrane becomes permeable to salts the corpuscle swells—slowly, since the excess osmotic pressure inside is but small. Higher concentrations of linolenic acid cause a further increase in the permeability of the membrane so that dianol brilliant blue can go in and, perhaps, hæmoglobin can come out. Still higher concentrations dissolve the membrane altogether and the hæmolysis is irreversible.

On the swelling hypothesis it is clear that after the addition of salt to a reversible preparation there should be no hæmoglobin in the suspending fluid, except, perhaps, for a small amount arising from the bursting of the corpuscles with a low resistance. This is easily tested by centrifuging off the corpuscles brought out by the addition of salt to a reversible preparation and examining the supernatant fluid.

The experiments were done by placing 2 c.c. of blood in a reversible state in a small centrifuge tube, adding 0.5 c.c. of saturated KCl and centrifuging the whole immediately for 5 mins. at 10,000 revs./min. KCl was used since it could be made very concentrated and was already made up for electrometric work, but the results have been checked and confirmed by using a supersaturated solution of Na_2HPO_4 to which a little KH_2PO_4 had been added to bring it to the normal cH of blood. The resulting solution was strongly hypertonic so that there could be no doubt that all the cells that were present would be thrown down by the centrifuge. 1 c.c. of the supernatant fluid was pipetted off, the rest shaken up and compared colorimetrically with the supernatant fluid. From this could be calculated (see p. 59) the proportion of corpuscles which had discharged their hæmoglobin and, hence, the volume which those remaining should have, apart from the volume, if any, possessed by the ghosts. A control was done with normal blood in which it was found that the sediment had a relative volume of 40.5 p.c., while the hæmatocrite reading gave 39 p.c. The other readings have been corrected accordingly.

It will be seen from the table that the actual volume of the sediment obtained was not much less than that of normal blood, but that it contained very little more hæmoglobin than did the supernatant fluid. In fact, it was not found possible to obtain a sediment containing much hæmoglobin without reducing the amount of the hæmolytic agent to

¹ I am indebted to Prof. A. V. Hill for this suggestion.

such an extent that the preparation was obviously not laked. Even then there was always some hæmoglobin in the supernatant fluid. Conversely, it was not found possible to obtain a preparation in which the

Laking agent	β or %	α observed	α calc. from colour	α of original blood
Water	.75	.34	.07	.355
	.74	.34	.07	—
	.70	.24	.13	—
	.62	.29	.13	—
	.57	.20	.12	—
	.74	.20	.13	.39
	.59	.30	.14	.25
	.55	.27	.13	—
Linolenic acid	.30	.15	.03	.385
	.35	.15	.01	—
	.32	.10	.01	.39

α in every case denotes the ratio of the volume of the corpuscles to the volume of the whole blood.

supernatant fluid contains as much hæmoglobin as the sediment since there is always a certain fraction of highly resistant corpuscles; moreover the stromata, in shrinking, would trap a certain amount of hæmoglobin and carry it down with them.

Suppose the ghosts had a volume $2\frac{1}{2}$ times the volume of the original corpuscles and contained the same concentration of hæmoglobin as the surrounding fluid. Then, if on shrinking to their normal size they became more or less impermeable to hæmoglobin they might contain finally a hæmoglobin concentration some 2 to 3 times that of the external fluid.

In fact in preparations with a β value between .5 and .6 there were often two distinct layers of sediment after centrifugation, the one dark and the other nearly the same colour as the supernatant fluid. The first, presumably, consisted of corpuscles which had never lost their hæmoglobin and the rest of ghosts. Careful observation showed that this layer of ghosts really possessed a rather higher concentration of hæmoglobin than did the supernatant fluid.

These results show that the swelling hypothesis in a simple form as stated above is untenable, but they also show that the re-adsorption theory of Brinkman and Szent-Györgyi is equally untenable; it is clear that in the preparations used the hæmoglobin left the corpuscles and did not return to them, except in the small amounts which can be accounted for by the presence of highly resistant corpuscles and by the shrinking of the stromata. It is suggested, therefore, that the optical heterogeneity of a reversed preparation of blood is due to the presence of ghosts, *i.e.* such a preparation consists of a suspension of colourless corpuscles in a solution of hæmoglobin.

It will be remembered that the microscopical observations showed that on increasing the β value or the linolenic acid concentration a stage was reached in which the corpuscular membrane was apparently permeable to dianol brilliant blue, even though the preparation was reversible. This may be regarded as additional evidence that the hæmoglobin has left the corpuscles. It seems probable, indeed, that when the conditions are such as to make the preparation homogeneous under the microscope, the greater part of the hæmoglobin has left the corpuscles.

The most noticeable change that occurs when blood is laked is a change in the colour which is most marked when the preparation is viewed by reflected light and on reversion of hæmolysis this colour change is reversed, the preparation becoming a bright scarlet instead of a very deep red. Moreover, a suspension of corpuscles in a solution of hæmoglobin is very much darker in colour than one in, say, a salt solution, and the more hæmoglobin there is in solution the darker is the colour. By means, therefore, of comparing the colour of a preparation of reversibly hæmolysed blood to which salt has been added with the colour of suspensions of corpuscles in solutions of hæmoglobin of various strengths, it is possible to obtain evidence as to whether the hæmoglobin is in (or on) the corpuscles or is in solution in the suspending fluid.

Control experiments having shown that the number of corpuscles per unit volume of the suspension did not affect the amount of light reflected by it to anything like the same extent as did the amount of hæmoglobin in solution, three suspensions were made up, one in Ringer, one in a hæmoglobin solution made by hæmolysing a portion of the blood in use, and one in a hæmoglobin solution of half this strength.

If, therefore, the blood preparation after reversion has the same appearance as the first of these suspensions, it indicates that all the hæmoglobin is in the corpuscles, and if it has the appearance of the second, it indicates that none is in the corpuscles.

Test-tubes containing these suspensions were photographed by reflected light along with a preparation of reversibly laked blood to which a small amount of saturated KCl had been added immediately before exposure. Figs. 7 and 8 show the result and in the table are given the relative densities of the images on the photographic plate in arbitrary units. It should be remembered, however, that these figures are only very approximate since the intensity of the reflected light is not directly proportional to the hæmoglobin concentration in the solution nor is the density of the image on the plate directly proportional to the intensity of the light falling on it. However, they can be considered as indicating

that roughly half the total hæmoglobin was in solution in the serum of the preparation in which hæmolysis had been reversed (in the case of linoleic acid rather less than half). The density figures for one preparation must not be compared with those for the other as they were taken on different plates.

Laking agent	"Reversibly" hæmolyse	100 % hæms.	Standards 50 % hæms.	No hæms.
Water $\beta = .55$	175	125	173	220
Linolenic acid = .30 %	200	125	177	285

In both these preparations the laking agent was in low concentration and corpuscles were distinctly seen under the microscope so that the results may be considered to be in line with those given previously. With higher concentrations of laking agents the figures would have still less meaning owing to the rapidity of re-hæmolysis.

This difficulty was obviated, however, by making use of one of the apparatus designed by Hartridge and Roughton⁽⁶⁾ for the measurement of the velocity of chemical reactions, which they were kind enough to lend me. Blood brought into the reversible state by water or linolenic acid is run into one of the systems of mixing jets and 10 p.c. NaCl into the other, so that in the observation tube we could observe the appearance of the reversed blood before it had time to re-hæmolyse. Incidentally it may be remarked that the velocity of reversion was very rapid so that all the blood that came out of the mixing chamber was reversed, while the re-hæmolysis did not take place to any extent while the blood was in the tube. The observation tube was placed in a strong beam of light along with tubes of standard suspensions of corpuscles in hæmoglobin solutions of various strengths, as described above, and the colour of the blood in it compared with the colours of the standards.

As was expected, it was found that a preparation with a β -value of .45 had the appearance of a suspension of corpuscles in a hæmoglobin solution corresponding to about 30 p.c. hæmolysis (very great accuracy cannot be attained in this method), while the colour of a preparation in which $\beta = .82$ corresponded to a hæmolysis of 100 p.c. An intermediate preparation with a β -value of .62 was found to have about 75 p.c. of its hæmoglobin in solution after the addition of salt. Thus these observations confirm the conclusions already arrived at in every respect.

If reversibly hæmolyse blood contains a large number of swollen corpuscles, it should possess a high viscosity and in fact it has been found that while the viscosity of irreversibly laked blood is less than that of normal blood, that of reversibly laked blood is always much greater (up to four times).

On the assumption that the action of water is solely one of swelling under osmotic influences, the viscosity of the reversibly laked blood can be calculated from the β -value and the percentage volume of the corpuscles, if we assume Hatschek's formula for the viscosity of emulsoids whose disperse phase occupies more than 60 p.c. of the total volume(3). Trevan(4) has found that this formula holds for bloods with a corpuscular volume of more than 45 p.c. so that the viscosities of our reversibly laked bloods should approximate to the theoretical values as we increase the hypotonicity of the suspending fluid. (The calculation is given on p. 60.)

Fig. 9 gives the values of the viscosities of a series of preparations plotted against the β -values, together with the theoretical curve. It will be seen that the experimental curve approaches the theoretical one up to a point and then sharply diverges from it. This is probably due to the commencement of stromatolysis of the less resistant corpuscles.

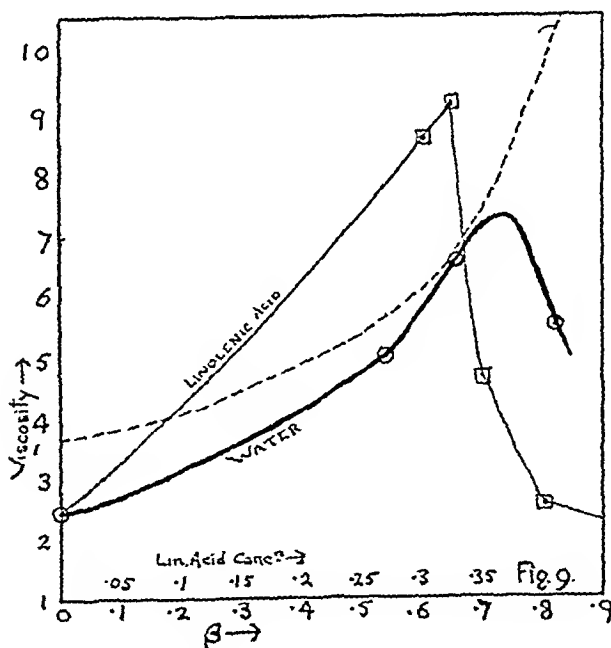


Fig. 9. Curves showing the relation between viscosity and β -value, theoretical, dotted line, experimental, thick line, and the relation between viscosity and linolenic acid concentration, thin line. The viscosities are expressed relative to those of the suspending fluids.

This figure also gives the viscosities of some preparations made with linolenic acid, plotted against the concentration of the acid, and it will

be seen that when the concentration is greater than $\cdot 4$ p.c. the viscosity of the laked blood is less than that of the normal blood, but that a smaller concentration gives preparations with very large viscosities indeed—up to nine or ten times that of the suspending fluid. This presumably indicates swelling of the corpuscles. Concentrations of linolenic acid greater than $\cdot 5$ p.c. also give preparations with high viscosities but this is due to a protein precipitation which can be seen under the microscope.

On Brinkman and Szent-Györgyi's theory the structure of laked reversible blood would be one of a suspension of ghosts of the same size as corpuscles in a solution of hæmoglobin, such as would be given by complete hæmolysis and the viscosity of such a suspension can be easily calculated. Normal blood has a viscosity of about $2\cdot 5$ times that of the serum and completely hæmolysed blood one of about the same or rather less, so that the viscosity of our hypothetical suspension would lie between $5\cdot 5$ and 6 times that of the serum. (Dilution of the serum by added water does not affect these figures.) In point of fact hæmolysed reversible blood may have a viscosity of $9\cdot 2$ in the case of linolenic acid and $6\cdot 6$ in the case of water.

It may be noted that on Hatschek's⁽³⁾ theory a blood with a relative corpuscular volume of $\cdot 77$ would have a viscosity of $9\cdot 2$; this would require a swelling of less than $2\frac{1}{2}$ times on the part of the corpuscles so that a simple change of shape from the biconcave to the spherical would be sufficient to account for the viscosities observed.

After reversion the viscosity returns to very nearly the same value as that of the original blood, as might be expected. During the subsequent spontaneous re-hæmolysis the viscosity rises but does not, as a rule reach its initial value partly owing to the dilution by the salt solution added and partly owing to the stromatolysis which always results on dilution of reversibly hæmolysed blood.

None of the evidence presented above can be said to support the adsorption theory and there are, moreover, several intrinsic improbabilities in it.

(1) The fact that the addition of any salt or even cane sugar is sufficient to cause reversal of hæmolysis indicates that the phenomenon is an osmotic one; it is not usual for an adsorption process to be influenced by such diverse agents.

(2) A calculation made by A. V. Hill shows that in order to pack the requisite amount of hæmoglobin on the surface of each corpuscle, there would have to be a layer some 50 molecules thick. No forces known

could retain a layer of such a thickness, especially with molecules of so enormous a size.

It may also be pointed out that the observations on the electrical resistance of reversibly hæmolyzed blood given by Brinkman and Szent-Györgyi can be equally well explained by the swelling of the corpuscles under the influence of the linolenic acid.

Moreover, the experiments of Bürker(5) quoted by them, in which he finds that the amount of hæmoglobin per corpuscle is proportional to the *surface area* of the corpuscle is not necessarily evidence that the hæmoglobin is held by surface forces but only that all the corpuscles used by him had the same thickness; this might be expected from teleological considerations, since the oxygen could not diffuse sufficiently rapidly through more than a certain thickness of hæmoglobin solution. It is worth noting, too, that he used only mammalian blood. Ponder(7) has shown, also, that the errors inherent in the measurement of the diameter of a red blood corpuscle are too great to permit one to decide whether the amount of hæmoglobin per corpuscle depends upon its surface area or its volume.

The most probable explanation of the phenomenon of reversible hæmolysis, then, seems to lie in a swelling of the corpuscles under the influence of the hæmolytic agent; reversion is due to a shrinkage of these corpuscles and they may or may not contain hæmoglobin, according as the degree of swelling has or has not been sufficient to cause the membrane to become permeable to hæmoglobin. In either case the solution would be optically heterogeneous.

SUMMARY.

(1) The phenomenon of reversible hæmolysis has been studied from various points of view with the object of determining the condition and location of the hæmoglobin both before and after reversal of hæmolysis.

(2) Bloods which are in the condition to show the reversal phenomenon can be divided into two classes which merge into one another, (a) those in which the laking agent is weak and (b) those in which it is strong.

(a) These consist of tightly-packed swollen corpuscles and are not completely transparent owing to the varying degrees of swelling in various corpuscles. Reversion consists in the shrinkage of the corpuscles back to their normal size. No hæmolysis, strictly, has taken place.

(b) In these the hæmoglobin has been discharged as a result of an excessive swelling of the corpuscles, but the stroma material has not dissolved and retains its structure. These preparations are completely

transparent and indistinguishable in their appearance from hæmolyzed blood. Reversal consists in the shrinkage of the stromata to their original size and the amount of hæmoglobin in them is only slightly greater than that in the suspending fluid.

(3) Reversal in the first class is complete and permanent; in the second class but little hæmoglobin is carried down with the disperse phase after reversal, and the optical heterogeneity is transient.

(4) These two classes are ideal; in any given case, owing to the varying resistance of the corpuscles, the effect observed partakes somewhat of the characteristics of both.

(5) There is no necessity for assuming a reversible adsorption of the hæmoglobin by the corpuscles.

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APPENDIX.

CALCULATION OF THE PROPORTION OF CORPUSCLES WHICH HAVE LOST THEIR HÆMOGLOBIN, FROM THE COLOUR OF THE SUPERNATANT FLUID.

Let c_s be the hæmoglobin concentration in the serum,

c_0 be the total hæmoglobin concentration,

α be the relative volume of the corpuscles in the original blood,

R be the ratio of the colours of the supernatant fluid and of the whole system as observed in the colorimeter,

v_0 be the volume of blood centrifuged

Then, since 1 c.c. of supernatant fluid is removed before shaking up,

$$c_s = R \cdot \frac{c_0 v_0 - c_s}{v_0 - 1},$$

$$\frac{c_0}{c_s} = \frac{v_0 - 1 + R}{R v_0}$$

Now suppose that in this blood a proportion p of the corpuscles originally present have discharged their hæmoglobin so that as far as their colour is concerned they have become part of the serum

Then the relative volume of the serum is now $1 - \alpha + p\alpha$

But, since we have not all the hæmoglobin in the serum but a portion p , we have

$$\frac{c_s}{c_0} = \frac{1}{1-a+pa} \cdot p,$$

or

$$p = \frac{1-a}{\frac{c_0}{c_s} - a}.$$

$(1-p)a$ is given in the table on p. 53 as " a calculated from colour."

CALCULATION OF THE VISCOSITY OF A SUSPENSION OF BLOOD CORPUSCLES IN A HYPOTONIC SOLUTION.

Take a volume V_0 of blood, of which $\alpha_0 V_0$ is corpuscles and $(1-\alpha_0) V_0$ serum.

Replace a volume V_s by water so that the dilution of the remaining serum is

$$\frac{(1-\alpha_0) V_0 - V_s}{(1-\alpha_0) V_0} = 1 - \beta.$$

Let

$$\left. \begin{array}{l} s_c = \\ s_s = \end{array} \right\} \text{total solids in } \left\{ \begin{array}{l} \text{corpuscles} \\ \text{serum} \end{array} \right.$$

and

$$\left. \begin{array}{l} w_c = \\ w_s = \end{array} \right\} \text{water content of } \left\{ \begin{array}{l} \text{corpuscles} \\ \text{serum.} \end{array} \right.$$

Then in the original blood since the osmotic pressure is the same inside and outside the corpuscles

$$\frac{s_c}{w_c} = \frac{s_s}{w_s},$$

and in the diluted blood for the same reason

$$\frac{s_c}{w_c + x} = \frac{s_s(1-\beta)}{w_s - x},$$

a volume x of water having entered the corpuscles and swollen them.

Whence

$$x = \frac{w_c w_s \beta}{w_c + w_s(1-\beta)}.$$

Put $w_s = (1-\alpha_0) V_0$ and $w_c = 7\alpha_0 V_0$ (to allow for the volume of the solutes in the corpuscles).

Then if a is the new corpuscular volume,

$$\begin{aligned} a &= \frac{\alpha_0 V_0 + x}{V_0} \\ &= \alpha_0 + \frac{7\alpha_0 V_0 (1-\alpha_0) V_0 \beta}{7\alpha_0 V_0 + (1-\alpha_0) V_0 (1-\beta)} \cdot \frac{1}{V_0} \\ &= \alpha_0 \left(1 + \frac{7\beta (1-\alpha_0)}{1 - 3\alpha_0 - \beta (1-\alpha_0)} \right), \end{aligned}$$

and if η is the viscosity of this suspension relative to that of the suspending fluid,

$$\eta = \frac{\sqrt[3]{\frac{1}{a}}}{\sqrt[3]{\frac{1}{a}} - 1}.$$

THE MAXIMUM FREQUENCY OF REFLEX RESPONSE
IN THE SPINAL CAT. BY SYBIL COOPER (*Yarrow
Student of Girton College, Cambridge, and George Henry Lewis
Student*) AND E. D. ADRIAN.

(*From the Physiological Laboratory, Cambridge.*)

THE experiments described in this paper form an extension of the attempt made by Adrian and Olmsted(1) to measure the refractory period in the central part of a reflex arc, the object being to obtain some of the numerical data needed for an understanding of central conduction. We have found that the conclusions drawn by Adrian and Olmsted have to be modified in one important particular and this has involved a re-examination of several related points. One of their methods consisted in stimulating a sensory nerve with a rapid series of break shocks and recording the electric responses of the muscle contracting reflexly. They found that in the spinal cat the tibialis anticus would give a regular sequence of electric responses as rapidly as 160 a second in response to stimuli of the same frequency applied to the popliteal nerve; with stimuli at 200 a second the response of the muscle became irregular after a few hundredths of a second and with higher rates it was always irregular. Since the same muscle stimulated by its motor nerve would respond as rapidly as 320-400 a second, they concluded that the maximum frequency of reflex response was determined by some factor in the central part of the reflex arc which limited the frequency of activation of the motor neurones to 160 a second.

Last summer Beritoff(2) announced that in the semitendinosus of a spinal or decerebrate cat he had found regular responses at a frequency as high as 250 and often 300 a second produced by reflex stimuli of the same frequency. We have therefore re-examined the response of the tibialis anticus and other muscles with various changes in technique, and we find that the value given by Beritoff must be accepted as correct, the lower value given by Adrian and Olmsted depending partly on a different method of assessing the "maximum frequency" and partly on a faulty method of recording the electric response.

Method. Our experiments have been made on spinal cats decapitated by Sherrington's method. Shielded stimulating electrodes are applied

to the popliteal branch of the sciatic and shocks are delivered at the requisite frequency by a rotating contact breaker connected with a coreless induction coil. Strengths of stimuli are expressed as reciprocals of the resistance in the primary circuit, unit strength corresponding to 1500 ohms, and also as multiples of the threshold stimulus. The nerves to the hamstring muscles are cut unless the response of the semitendinosus is to be investigated, but other nerves are left intact and the tendon of the muscle is left attached to its insertion. The temperature of the muscle under investigation is measured by a needle thermojunction of the form described by Adrian and Watts(3). The chief points in which our methods differ from those of Adrian and Olmsted are concerned with the recording of the electric response of the muscle. The string tension used by them was such that .001 volt through the string (2500 ohms resistance) gave a deflection of $7\frac{1}{2}$ to 10 mm. at a magnification of 440. This tension appeared to be great enough for accurate recording, since the string would give a regular series of oscillations at frequencies as high as 400 a second when a series of break shocks were sent through it from the stimulating apparatus. But a string might be able to follow a simple periodic oscillation of high frequency and yet be unable to give a true rendering of the more complex action currents in a reflex. In the response of the tibialis anticus there are irregular variations in amplitude as soon as the stimulation frequency reaches 160-200 a second, and we now find that a string at 10 mm. tension will give the impression of an irregular frequency of response when a tighter string will show a regular sequence of small waves superimposed on a slower, irregular series. In the present work we have used a tension of 5 mm. or 2.5 mm. for .001 volt. Condenser damping was sometimes used at the higher tension to make the string aperiodic. Since an increase of tension above 5 mm. does not lead to the appearance of any greater frequencies in the record but merely produces a reduction in size, we conclude that this tension gives a faithful record of the highest frequencies in a reflex response. It must be admitted that the frequencies involved are only just within the accurate range of the instrument according to Fahr's analysis(4).

The action currents are led from the muscle by electrodes of stout silver wire thrust into its substance. Before use the surface of the wire is roughened with a file and silver chloride is deposited on it electrolytically. Some of this is rubbed off when the wire is introduced into the muscle, but enough remains to prevent very rapid polarisation when a constant current is passed (see calibration curve, Fig. 1).

Maximum frequency of response to reflex stimulation. Fig. 2 shows typical records of the response of the tibialis anticus to high frequencies

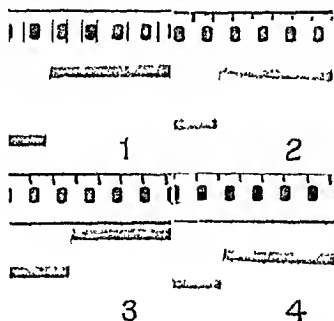


Fig. 1. Calibration curves of string galvanometer. Silvered glass string
4 microns diam., resistance 2500 ohms.

1. Tension 5.5 mm. per nullvolt. 2 mv. through string alone.
2. Ditto with electrodes and muscle in circuit.
3. Tension 3 mm. per nullvolt. 2 mv. through string alone.
4. Ditto with electrodes and muscle in circuit.

Time marker (vertical lines at top of film) gives 0.2 sec. intervals in all records.

of reflex stimulation. It will be seen that irregularities of amplitude are present with frequencies of 200 a second but that the intervals between the oscillations are still regular with stimuli at 240 a second. When the reflex excitability of the preparation is good, a regular sequence of responses at 240 a second may last for $\frac{1}{2}$ second or more, but with continued stimulation the response eventually becomes irregular. With stimulation at 280 and at 320 a second the irregularity appears much sooner and there may be small groups of responses at 280 or 320 a second separated by groups with no definite rhythm. To produce these high frequencies of response we have used stimuli from 5 to 10 times the threshold strength. A further increase in strength up to 50 or 100 times the threshold does not increase the frequency which can be attained, though a reduction below 5 may diminish it. We have not examined the relation between strength and frequency in any detail, but it seems to be in general agreement with what we should expect from the form of the recovery curve for mammalian nerve.

The only difference between these results and those of Beritoff is

nerve and we find that at 37–38° C. the muscle will usually follow a rhythm of 400 a second and occasionally one of 480. These frequencies are rarely maintained for more than $\frac{1}{2}$ to $\frac{1}{3}$ sec. and sooner or later the responses drop to half the original frequency; usually passing through a stage where alternate responses are large and small. But with stimuli at 360 or 320 the muscle will continue to follow the rhythm for several seconds at least. With reflex stimulation, on the other hand, the same muscle at the same temperature will rarely follow a rhythm of 320 a second except for occasional short periods at the beginning of a contraction, and even at 240 the responses become irregular after one or two seconds. The point is illustrated in Fig. 2, which gives the response of the same muscle to reflex and motor nerve stimulation.

Again, if the muscle is stimulated by its motor nerve at a frequency too great for it to follow (500 a second or more) or if it has been stimulated at a lower rate until fatigue has set in, the response will generally consist of a regular series of action currents occurring at half the frequency of the stimuli. In a reflex we have never observed responses at half the frequency of stimulation; when the frequency is not followed the responses occur irregularly at a rate slightly less than the maximum regular frequency. If the motor side of the reflex arc is transmitting impulses at the frequency of stimulation and the failure is due to the muscle, it is difficult to see why the frequency of response is never halved as it is when the motor nerve is stimulated directly.

These arguments are both of some value, but further proof is needed before we can be certain that the failure lies in the central paths and not in the muscle.

Frequency of response in motor nerve with reflex stimulation. The most direct way of investigating the question would be to record the electric responses of the motor nerve during reflex stimulation instead of recording those of the muscle. We have made several attempts to do this but the technical difficulties have been too great. It is a relatively simple matter to record single reflex electric response in the motor nerve with a string at 10–20 mm. tension, but difficulties begin to arise when the responses follow one another at very short intervals. The chief problem is to eliminate the slight spread of the stimulating current into the high resistance circuit of the nerve and galvanometer. If the string is tight enough for accurate recording, the reflex responses are very small, and at high frequencies we have found it almost impossible to disentangle the true action currents from the small artefact due to current spread (or capacity effect) from the stimulating circuit. We have therefore aban-

done the attempt in favour of a less direct, but technically simpler method.

Effects of change of temperature in the spinal cord. The foregoing results suggest that the motor neurones concerned in the flexion reflex cannot be made to send out a regular series of impulses at a frequency greater than 250-300 a second. If this is so when both spinal cord and muscle are at 38° C., a fall of temperature limited to the cord ought to reduce this frequency. If, on the other hand, the cord at 38° can give a regular series of discharges as often as, say, 500 a second, then cooling the cord will not begin to affect the maximum frequency of response in the muscle until a much lower temperature has been reached. To test this point we have made a series of experiments with the tibialis anticus kept throughout at 38° and the spinal cord cooled to various temperatures between 38° and 28°.

A spinal preparation in good condition will usually give a fairly strong reflex although its rectal temperature has been allowed to fall to 28° C. In the earlier experiments the heating of the table was turned off and the whole body of the cat was allowed to cool, the leg being kept warm by a hot-water bottle or a lamp. The temperature of the muscle was measured by a needle thermo-junction and that of the cord was assumed to be equal to the rectal temperature. As the temperature of the body falls very slowly, we changed the method in the later experiments and cooled the cord more rapidly by shaving the skin over the lumbar region and covering it with small lumps of ice. A needle thermo-junction was thrust into the back muscles by the side of the vertebral column so that the junction was at the same depth below the surface as the spinal cord. The temperature measured by this method was found to agree within one degree with that given by a junction in the cord itself. When the cord had reached the required temperature the sensory nerve was stimulated at various frequencies and the response of the muscle was recorded in the usual way. Five experiments were made and their results are given in Table II. In the last two, after the reflex observations had been made with the cord at its lowest temperature, the stimulating electrodes were transferred to the motor nerve and the maximum frequency of response was determined with the muscle at 38° and then at lower temperatures.

All the experiments agree in showing a fall in the maximum frequency of response when the cord is cooled, and the actual values agree very well with one another. With the cord at 28-30° the maximum frequency lies between 160 and 200 in all the experiments and at 32-35°

TABLE II.

Exp.	Temperature		Reflex response. Maximum regular frequency			Stimulus to motor nerve.	
	Muscle	Cord				Temperature of muscle	Maximum regular frequency
6	39°	39°	280 +	320 ±	360 -		
	—	32		200 +	240 -		
7	41	33		240 +	280 -		
	40	38		320 +	360 -		
8	39	32		200 +	240 -		
9	38	39		280 +	320 -	37.5°	400 +
	—	31	160 +	200 ±	240 -	28	320 + 360 ±
10	38	38	280 +	320 ±	360 -	38	400 + 480 ±
	—	35		240 +	280 -	30	320 + 360 ±
	—	32		240 +	280 -	27.5	240 + 280 + 320 ±
	—	30.5		200 +	240 -		
	—	27.5		160 +	200 -		

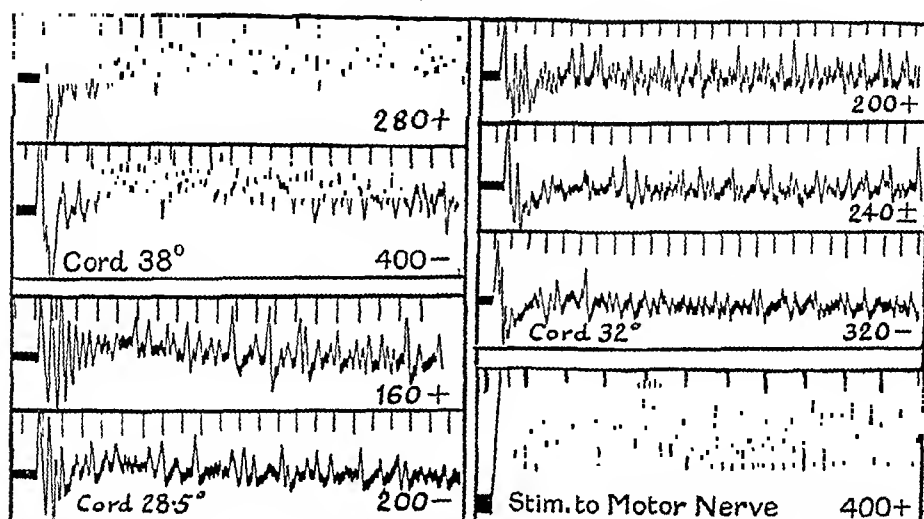


Fig. 3. Maximum frequency of reflex response with spinal cord at different temperatures, muscle at 38° C. throughout. String tension 5 mm. per mv.

Cord 38°, stim. 15 units (17 times threshold). Cord 32°, stim. 15 units (9 times threshold).

280 per sec. regular.

200 per sec. regular.

400 per sec. irregular.

240 per sec. doubtful.

Cord 28.5°, stim. 30 units (13 times threshold).

320 per sec. irregular.

160 per sec. regular.

Stimulus to motor nerve. 15 units (12 times threshold).

200 per sec. irregular.

400 per sec. regular.

it lies between 200 and 240. Records from the last and most complete experiment are shown in Fig. 3, together with a record obtained subsequently by stimulating the motor nerve. This has been enlarged to show the very high frequency which can be followed by the muscle.

These experiments show that cooling limited to the spinal cord

causes a reduction in the maximum frequency of reflex response in the muscle, but caution is needed before we draw more detailed conclusions. In the first place the reduction may have been due to the lengthening of the refractory period of the motor and sensory fibres in the spinal roots. This possibility may be dismissed at once. When the muscle itself is cooled to 27.5° C. and stimulated by way of the motor nerve, the maximum frequency of response is still as high as 240 a second. That of the nerve cannot well be less than this and will be almost certainly greater, so that the maximum reflex frequency of 160-200 with the cord at 28° cannot be due to the cooling of peripheral nerve fibres. Neither can it be due to the inability of the muscle to respond at a higher frequency, for the muscle is kept at 38° and is able to respond at 400 a second to motor nerve stimulation. Evidently the limit of 160 a second is imposed by the central nervous system when the cord has been cooled. When the cord is at its normal temperature, however, the maximum frequency of discharge will naturally be greater than 160 a second and it may then surpass the ability of the muscle to respond. We have therefore to decide what will be the maximum frequency of discharge when the cord is at 38°, granting that the frequency at 28° is 160 a second. We are handicapped by the lack of exact knowledge of the temperature coefficient of the recovery process in the spinal centres, but direct measurements of the coefficient can be made in the case of the muscle and it is probably safe to assume that much the same value will hold good for the cord. Table III gives the results of a series of experiments in which the motor nerve is stimulated and the maximum

TABLE III.

Exp.	Temperature of muscle	<i>Stimulus to motor nerve.</i>		
		Maximum regular response		
2	40°	360 +	400 ±	
9	34.5	360 +	400 ±	
	28		320 +	360 -
10	38	400 +	480 ±	640 -
	30	320 +	360 ±	640 -
	27.5	240 +	280 ±	320 ±
11	39		400 +	
	29		320 +	360 -
12	33.5		400 +	440 -
	40		560 +	
13	30.5		320 +	360 -
	38		400 +	440 -
	29.5		320 +	360 -
	35		360 +	400 -
	40		440 +	480 -
	30		320 +	360 -
	25	240 +	280 ±	320 -

frequency of regular response (defined as before) is measured at different temperatures. Fig. 4 shows the measurements in Exp. 13 and it will be seen that a rise of temperature from 30–40° increases the maximum

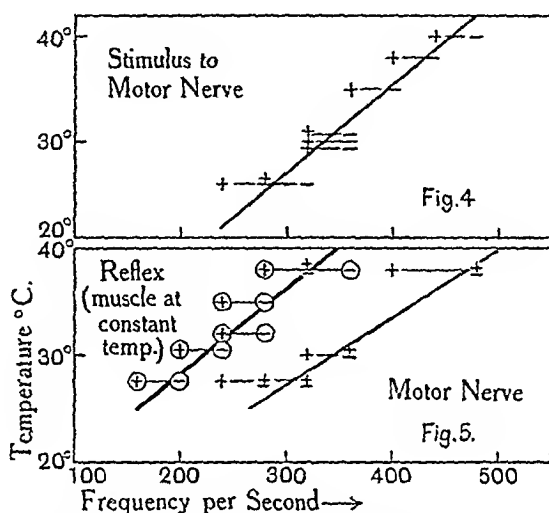


Fig. 4. Effect of change of temperature on maximal frequency of response in tibialis anticus to stimuli applied to motor nerve. Stimuli 27 units strength (7.3 tin threshold). Exp. 13.

Fig. 5. Effect of change of temperature of spinal cord on maximal frequency of reflex response, the muscle remaining at 38° throughout (Exp. 10). The curve on the right gives the maximum frequency for the muscle at different temperatures with motor nerve stimulation.

frequency from about 320 to 440. This gives a coefficient of about 1 for a ten degree rise between 30 and 40°. If we take this as applying the maximum frequency of discharge from the cord, and if the frequency at 28° is 160–200 a second, the frequency at 38° should be 220–280 second—and this agrees fairly well with the observed maximum frequency of reflex response in the muscle when the cord is at 38°.

Suppose, however, that the low temperature imposes an abnormal slow rate of discharge because it interferes with the function of the cord in some way over and above that due to the slowing of the recovery processes. The temperature coefficient might then be considerably higher than 1.4 and the cord at 38° might really be able to discharge as rapidly as e.g. 500 a second, though the muscle would be unable to follow this rate. If so, we ought to find that cooling the cord from 38° will cause a change in the maximum frequency of the muscle response until the temperature has fallen to such an extent that the cord can no longer discharge too rapidly for the muscle to follow, and further cooling below

this temperature will produce a sudden reduction in frequency. The curve relating the temperature of the cord to the maximum frequency of response ought therefore to show a discontinuity somewhere between 28° and 38° . In Fig 5 the results of Exp 10 are shown graphically and there is evidently no such discontinuity. The maximum frequency of the reflex response increases in a perfectly regular way with the temperature of the cord. The rate of increase is slightly greater than in the case of the muscle at different temperatures stimulated by the motor nerve, but the difference is not outside the range of error. It seems clear, therefore, that the value of 210–320 a second for the maximum frequency of reflex response with the cord at 38° represents the maximum frequency of discharge from the cord and not the maximum frequency which the muscle can follow.

A comparison of the two curves in Fig 5 shows that if the temperature of the whole animal is reduced, the maximum frequency in the reflex is always slightly less than the frequency which the muscle can follow at that temperature. The difference is not great, and if the muscle alone were cooled it might cease to be able to respond to the highest frequency which the cord can send to it. In a cat which has just been anaesthetised the temperature in the substance of the tibialis anticus has been as low as 31°C although the rectal temperature was 38.5° , but even with this difference the muscle should still be able to follow the highest rate of discharge from the cord.

Frequency of response with other reflex arcs. Beritoff has shown that the maximum reflex frequency is about 300 a second in the flexors and in the extensors of the thigh and our own experiments now give the same value for the tibialis anticus. We have also tried the effect of stimulating the internal saphenous instead of the popliteal and have found the same maximum frequency in the tibialis anticus. In four experiments we have stimulated the descending tracts of the spinal cord by small bipolar electrodes thrust into the exposed cross section of the cord in the cervical region. The effect of stimulation is to produce sometimes flexion and sometimes extension in the legs and the galvanometer leads are placed on whichever muscle is most active. With frequencies of stimulation below about 80 a second the contractions are often feeble and the responses of the muscle may be irregular. With higher frequencies (80–160 a second) the rhythm of the stimulus generally appears on the muscle response, though a complete lack of any regular frequency is not uncommon. The highest regular frequency we have recorded is 280 for occasional short periods and 240 for $\frac{1}{2}$ sec or more,

the muscle being the gastrocnemius. Destruction of the cord in the cervical region put a stop to the responses, so that the stimuli presumably took effect on the descending tracts in the cervical region and did not spread down the cord. These experiments are too few to generalise from, but they suggest that the motor centres for the hind limb muscles, through whatever paths they are activated, will not send out a regular succession of impulses to the muscles at a frequency greater than 240–320 a second. There is, of course, the alternative possibility that 320 a second is all that the muscles can do, but in one muscle at least, namely, the tibialis anticus, our results show that motor nerve stimulation will give definitely higher rhythm.

“Repetitive Discharge” from the spinal cord. Forbes, Ray and Griffith⁽⁵⁾ have raised the possibility that in response to a single afferent stimulus each neurone of the motor centre may discharge, not a single impulse, but a succession of impulses occurring at so high a frequency that the muscle can only respond to the first of the series. If each stimulus produces a group of these high frequency discharges, the response to reflex stimulation in the motor nerve and in the muscle might be represented graphically as in Fig. 6.

If this suggestion is correct the maximum frequency of regular response in the muscle may depend, not on the maximum frequency with which the cord can discharge impulses, but on the duration of each group which it discharges—for the muscle response will become irregular (if it does not cease altogether) as soon as the successive groups coalesce.

There is no doubt that under certain conditions a single impulse in the afferent nerve may produce a multiple discharge in the efferent, for the electric response of the muscle may be multiple. In this case, however, the impulses in the motor nerve are obviously spaced in such a way that the muscle gives a succession of responses, and we have no

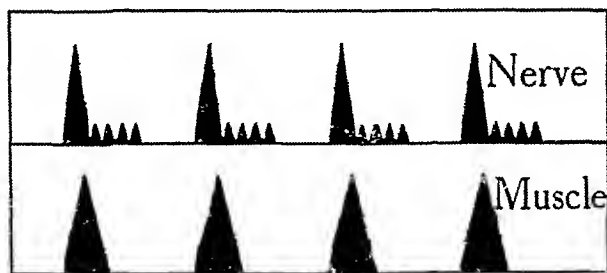


Fig. 6. For description see text.

direct evidence of a discharge of impulses from the cord at so high a frequency that the muscle only responds to the first of the series. That such a grouping of impulses determines the maximum frequency of reflex response seems to be ruled out by the experiments in Table II, where the cord was cooled. If the maximum frequency is fixed by the length of each group of impulses, as in Fig. 6, a reduction in frequency must be due to a greater duration of each group. The interval between the successive impulses in the group cannot be increased to any considerable extent or the muscle will give a multiple instead of a single response; thus the number of impulses in each repetitive discharge should increase when the cord is cooled. But cooling the cord does, in fact, reduce the tendency to repetitive discharge, or at any rate to that form of discharge which gives a multiple response in the muscle. This may be seen from Fig. 7, which gives the response of the muscle in



Fig. 7. Disappearance of "repetitive response" when cord is cooled.

Exp. 10. A. Spinal cord and muscle at 38° . Reflex response to stimuli at 17 per sec., 3 units strength.

B. Cord at 35° and muscle at 38° . Stimuli 17 per sec., 3 units.

Exp. 10 to reflex stimulation at 16 a second with the cord at 38° and at 35° . With the cord at 38° and stimuli 3.5 times the threshold strength (3 units) the response consists of large "primary" waves at the frequency of stimulation with many small secondary waves between them. At the lower temperature with the same stimulus no secondary waves appear. The same disappearance of repetitive response in the muscle was observed in other experiments in which the cord was cooled.

Again, if we compare the flexion reflex in a number of spinal preparations we find that the duration of the repetitive response in the muscle with low frequencies of stimulation varies within wide limits. Adrian and Olmsted stated that very strong stimuli were necessary to produce repetitive responses, but later work has shown that this is

only true of preparations observed fairly soon after decapitation when the effects of shock and of the anæsthetic are probably still considerable. A preparation with brisk reflexes, observed some hours after decapitation will generally give a record like that of Fig. 7A, with stimuli only 5-10 times the threshold strength, but another, less active, may need much stronger stimuli before any secondary waves appear. Yet both will give the same maximum frequency of reflex response, and the length of time after decapitation makes no difference to the result. In the same way we find a much greater tendency to repetitive response in decerebrate as compared with spinal animals(6), but the maximum regular frequency in the reflex is the same in both. It is, therefore, highly improbable that this frequency depends on a particular grouping of repetitive discharges from the spinal cord.

Frequency of discharge with other forms of reflex stimulation. When a spinal cat carries out the flexion reflex in response to a mechanical stimulus such as pinching the skin of the foot, the electric response of the tibialis anticus consists of a series of oscillations, irregular in frequency and amplitude and occurring at an average rate of 150-200 a second. A response of this kind in the muscle might be produced by impulses discharged from the spinal centres at the same frequency as that appearing in the electromyogram, or at a frequency much too rapid for the muscle to follow. In the latter case the frequency of discharge from the cord would have to be well over 500 a second, for with lower frequencies the muscle would be likely to show a regular 2 : 1 response. The experiments already described suggest that the former explanation is correct, for the spinal centres appear to be unable to discharge at a frequency too great for the muscle.

The question needs further investigation, however, for it has been the subject of considerable discussion and it is vital to the interpretation of the electromyogram. In some recent work on the frog we have given a brief account of the main arguments involved(7). In this work we concluded that the frequency shown in the electromyogram was generally identical with the frequency of discharge from the cord, since there was little or no change in rate when the temperature of the muscle was altered within limits, provided that the temperature of the spinal cord remained unchanged. In these experiments the muscle was generally warmed above the general body temperature, since there was some danger that cooling would make it unable to keep pace with the discharge from the cord although it had been able to do so at normal temperature. In mammalian experiments the muscle cannot be warmed above 38° without

danger of damage from heat rigor, but if the rate of discharge from the cord is not higher than about 200 a second it ought to be possible to cool the muscle through 10° or more without interfering with its ability to respond to every impulse from the cord.

We have made three experiments in which reflex contractions were provoked by pinching the foot, the temperature of the tibialis anticus being varied from 22° to 40° C. Typical records of the electric response at different temperatures are given in Fig. 8. Evidently a change of 10° in

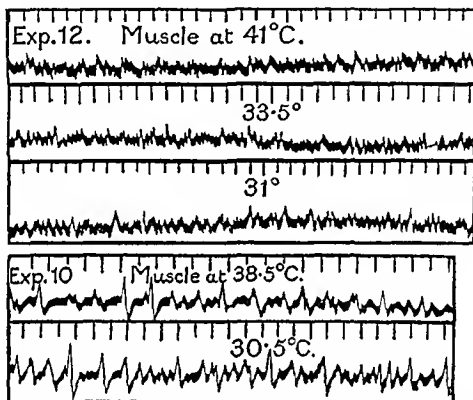


Fig. 8. Flexion reflex produced by pinching foot. Response of tibialis anticus. Temperature of cord remains constant and temperature of muscle is changed. The frequency of the responses remains unaltered. Tension 5 mm. in both experiments.

the muscle makes no obvious difference to the frequency of the reflex electromyogram, though the response is so irregular that the oscillations have to be counted over considerable periods before we can be certain. In Table IV the actual frequencies are given, the total number of oscillations being counted in periods of $\frac{1}{10}$ second taken at random from different parts of the record. There is no evidence that the frequency is altered by a change of temperature of 10° in the muscle, though the total number of oscillations in a second is reduced slightly when the muscle is cooled as low as 22° C.

To determine how great a change in frequency should occur if the muscle were responding to a series of excitations too rapid for it to follow, we have taken records from the cat's tibialis anticus, at different

TABLE IV.

Exp.	Temperature		Reflex response to pinching. Frequency per sec. in periods of $\frac{1}{10}$ sec.						Average
	Rectal	Muscle							
10	38°	32°	150	140	130	140	140	140	
	—	38.5	100	130	110	90	120	110	
	—	38	120	140	120	130	100	122	
	—	30.5	140	100	110	130	140	124	
	—	31.5	120	130	120	90	140	120	
12	38.8	40	190	180	210	180	180	188	
	—	33.5	200	190	200	180	170	188	
	—	41	190	150	180	170	210	180	
	—	31	170	180	190	180	190	182	
	—	40	160	200	180	170	180	178	
14	38.5	39	190	230	190	200	210	204	
	—	40.5	200	210	200	190	240	208	
	—	32	270	220	260	240	220	242	
	—	29	240	220	240	200	210	222	
	—	22	200	200	210	220	190	204	
	—	38	200	200	210	270	230	222	

temperatures, stimulated by the motor nerve at a frequency of 640–800 a second. The results are shown in Table V. The response of the muscle

TABLE V. Exp 13. Motor nerve stimulated at 640–800 a second.

Temperature of muscle	Irregular response in muscle. Frequency per second (average)
25°	220
29.5	220
30.5	200
35	270
38	300
40	320

was irregular in each case and the figures give the average frequency. This increases from 220 at 25° to 320 at 40°, giving a temperature coefficient of at least 1.3 for a change of 10°. A slightly higher coefficient is shown in Garten's results(8). Stimulating a rabbit's muscle nerve preparation with a constant current applied to the nerve, he found that the frequency of response in the nerve or muscle was about 100 a second at 25° and 180–400 a second at 37°. These results are not strictly comparable to those in Table V as a different type of stimulus is used, but it is probably safe to assume that a change from 28 to 38° would increase the frequency of the irregular response by at least 1.3 times. Table IV shows that in the reflex there is no such increase in frequency.

These records do not show that the spinal cord never discharges at a higher rate than that shown in the electromyogram, but merely that it does not do so as a rule. It is not uncommon to find small groups of four or five oscillations at a high frequency and these occur so irregularly that it is difficult to make sure whether their frequency is or is not

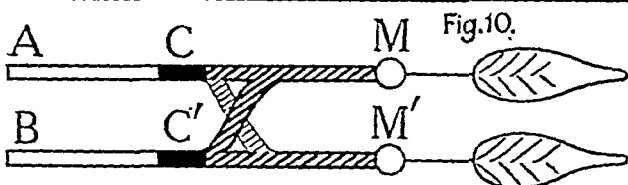
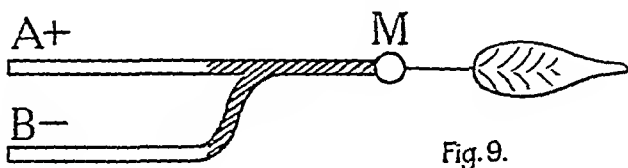
affected by a change of temperature of the muscle. Since the total number of oscillations in a second does not change appreciably, it is unlikely that there can be a change of frequency lasting for any appreciable fraction of the response. It is possible, therefore, that the frequency of discharge from the cord may occasionally become too fast for the muscle to follow, but by far the greater part of the reflex discharge must be composed of impulses with a frequency identical with that shown in the electromyogram.

DISCUSSION.

The chief interest of these results lies in the fact that the highest frequency of discharge from the cord is considerably smaller than the highest frequency of response in a motor or sensory nerve. The smallest interval between successive impulses leaving the cord lies between 0.04 and 0.03 second, whereas the smallest interval between successive impulses set up in a nerve by direct stimulation lies between 0.01 and 0.02 second. Presumably there exists somewhere in the grey matter of the cord a region which will only transmit impulses at 250-300 a second, although impulses at a higher frequency may be reaching it from the afferent part of the arc. The result will be that on the motor side of this limiting region the impulses will be so spaced out that each travels in a fibre which is well on the way to complete recovery after the passage of the previous impulse.

This conclusion has a bearing on the suggestion that the mechanism of central inhibition is comparable to that of Wedensky inhibition in a muscle nerve preparation. The basis of the Wedensky phenomena is that impulses recurring at a high frequency may fail to pass a region of decrement because the tissue will have no time for complete recovery between successive impulses and so each will be of small size and unable to travel far without extinction. A region so occupied by a rapid series of small impulses will be unable to transmit any other impulses which may reach it in addition to the inhibiting series. Fig 9 shows how this type of inhibition might occur in the central nervous system. *A* is an excitatory nerve and *B* an inhibitory, *M* is the group of motor neurones on which they take effect. The shaded region conducts imperfectly and part of it is common to both arcs. Assume that the decrement is so adjusted that impulses from *B* can never reach *M* whatever their frequency, whereas impulses from *A* can do so provided that the region is given time for complete or almost complete recovery between each impulse. If we stimulate *A* at a fairly low frequency, the impulses set up

will be able to pass through to *M*. If we stimulate *B* simultaneously, the impulses from *B* will not reach *M* but they will travel for some



Figs. 9 and 10. Scheme of central connections for excitatory and inhibitory arcs. The shaded areas conduct with a decrement and the black areas limit the frequency of impulses to 250-300 a second.

distance over the same path as the impulses from *A* and the overcrowding which results will reduce the size of the impulses from *A* and make them no longer able to reach *M*. Thus the stimulus at *B* will give rise to inhibition.

The likelihood of some such process actually taking place when an inhibitory nerve is stimulated has been greatly strengthened by the important work of Brücke(9) and of Beritoff(10). Brücke has shown that an impulse in the inhibiting afferent nerve has a maximum effect when it bears a definite phase relation to the impulse in the exciting afferent nerve, and Beritoff has shown that the inhibitory effect of each of a series of impulses lasts for about $\cdot 004$ second, so that there is a definite rhythm of inhibition corresponding to the rate of stimulation of the inhibitory nerve. Both lines of work suggest that the inhibitory effect of an impulse is closely connected with the refractory phase (absolute and relative) which accompanies it. If we assume that the inhibitory effect is due to the delay of recovery which the impulse will produce in the decremental region, we can calculate the recovery time of this region as follows. Since Beritoff finds the effect of each impulse lasting for $\cdot 004$ second it follows that the decremental region cannot take less than this time for complete recovery. Adrian and Olmsted found that the part of the arc upon which anaesthetics take effect cannot take more than $\cdot 006$ second for complete recovery, and in view of the present results this time should probably be shorter. A region which conducts imperfectly

under normal conditions is likely to be affected by anæsthetics in preference to any other part of the arc and we may therefore conclude that in the decremental region the time for complete recovery after the passage of an impulse is not less than $\cdot 004$ or more than $\cdot 006$ second.

Now it is clear that the simple mechanism shown in Fig. 9 is not enough. It accounts for the fact that stimuli at *B* always inhibit, but not for the fact that stimuli at *A* always excite. Since the impulses are extinguished in the region of decrement if they follow one another too closely, we ought to find that a rapid succession of impulses from *A* would fail just as much as a slower succession from *A* combined with impulses from *B*. Actually we find that in a normal preparation very rapid stimulation of an excitatory nerve is as effective as slow stimulation. To account for this we must add to the scheme a region somewhere on the afferent side of the region of decrement which will prevent the passage of impulses from *A* at too high a frequency. Suppose, for instance, that the region marked *C* (Fig. 10) will only allow impulses to pass through at such a frequency that the decremental region has time for complete recovery between each impulse; then stimulation at *A* can never lead to inhibition although combined stimulation of *A* and *B* may still lead to overcrowding and extinction.

It will be seen that the experimental results agree in a remarkable way with the requirements of this scheme. Our results show that somewhere in the reflex arc there is a region which limits the number of impulses passing to 250-300 a second. We may reasonably locate this at *C* in the figure. The least interval between successive impulses entering the decremental region from *A* must therefore be $\cdot 004$ – $\cdot 0033$ second. We have calculated the time for complete recovery in the decremental region as somewhere between $\cdot 004$ – $\cdot 006$ second. Thus the least interval between successive impulses from *A* is long enough to admit complete or almost complete recovery in the decremental region, and it follows that stimulation of the excitatory nerve is never likely to produce inhibition whatever the frequency of the stimuli.

In the diagram the inhibitory impulses from *B* are represented as having to pass through a similar filter *C'* before they join the path from *A* to *M*. It will make little difference to the result whether such a filter is or is not present, for the only essential is that the impulses from *B* should always be extinguished before they reach *M* and that for the final part of their career they should travel over the same path as that of the impulses from *A*. With such an arrangement a single impulse travelling from *C'* towards *M* should produce inhibition for $\cdot 004$ second.

To account for the more lasting inhibitory effect which may follow a strong single stimulus at *B* or a series of stimuli at low frequency, we have only to suppose that these produce a repetitive discharge of impulses in the inhibitory path from *C'* to *M* just as they do in the motor centres (*M'*) to which they lead. In fact the exact coincidence in the duration of the inhibitory and excitatory effects which result from stimulating an afferent nerve is readily explained if we suppose that the train of impulses passing from *C'* towards *M* is exactly similar to that passing from *C'* to *M'*.

The scheme of inhibitory and excitatory arcs shown in the figure differs in several respects from that given in Keith Lucas's book⁽¹¹⁾ and from the amplified form of this discussed by Forbes⁽¹²⁾; it has a closer resemblance to that given by Brücke. Our excuse for putting forward another modification with the same underlying principles is that we have now some of the numerical data which are needed to bring the scheme into closer relation with the facts of experiment.

SUMMARY.

Adrian and Olmsted stated that if the popliteal nerve of a spinal cat is stimulated rhythmically, the reflex response of the tibialis anticus shows rhythmic oscillations of the same frequency as the stimuli up to 100 a second. Beritoff has recorded a higher maximum frequency for the semitendinosus, namely, 250-300 a second, and we now find that this value applies also to the tibialis anticus, the lower figure depending partly on a different criterion of "maximum frequency" and partly on faulty galvanometric technique. The maximum frequency in our experiments ranges from 240 to 320 a second, though the latter value is only maintained for very short periods.

We have attempted to decide whether this maximum frequency is determined by some factor in the central part of the reflex arc or by the inability of the muscle to respond at a higher rate. We find that the muscle stimulated by its motor nerve will give a regular response at a frequency appreciably higher than that in the reflex, but the most conclusive evidence is given by experiments in which the temperature of the spinal cord is reduced whilst that of the muscle remains constant. This has the effect of reducing the maximum frequency of reflex response, though the ability of the muscle to respond at a higher frequency remains, of course, unchanged. We conclude that the maximum frequency of 240 to 320 a second represents the maximum rate at which the motor centre can discharge a regular succession of impulses.

The electromyogram of the flexion reflex evoked by pinching the foot shows irregular oscillations of an average frequency of 100-250 a second. These might be due to a discharge of identical frequency from the cord, or to one much too rapid for the muscle to follow (over 500 a second). If the latter interpretation is correct, a change of temperature in the muscle ought to change the frequency of the electromyogram, but we find that cooling the muscle through 10° C makes no appreciable change in frequency. We have, therefore, no evidence that the rate of discharge from each motor neurone can exceed 210-320 a second. This conclusion, taken in conjunction with Beritoff's work on inhibition, leads to a simple explanation of the difference between excitatory and inhibitory arcs.

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Paper by Lipschutz, Krause and Voss. VOL 58

On p. 164, line 5 from the top, omit this: "The passage should read: a retention of the testicle was caused without spermatogenesis (as we see) being necessarily inhibited."

THE EFFECT OF CARBON DIOXIDE ON THE RATE
OF RECOVERY IN NERVE. BY SYBIL COOPER,

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of Girton College, Cambridge.*

(From the Physiological Laboratory, Cambridge.)

THE present research is the outcome of an attempt to analyse the effect of carbon dioxide on the conductivity and excitability of nerve by the methods used by Keith Lucas (1) for studying the effect of alcohol, and by the present writer (2) for the effects of asphyxia. Lucas' method depends largely on measurements of the least interval for muscular summation and its advantage lies in the fact that it distinguishes changes in the conductivity of the nerve (or in the decrement suffered by the impulse in its passage through the affected area) and changes in the rate of recovery (or the duration of the refractory state). In the case of alcohol, Lucas showed that the conductivity of the nerve might be completely suspended without any change in the duration of the refractory state. The least interval for muscular summation would increase as the decrement increased, but the "recovery time" would remain unchanged. As the recovery is complete in $\cdot 015$ second, the least interval for muscular summation would never exceed this value, because the second impulse would then be as large as the first and as capable of passing through the region of decrement.

The early work of Grünhagen and Fröhlich suggests that carbon dioxide acts in a similar way to narcotics such as ether or alcohol and it was expected that, as with alcohol, the least interval would not exceed $\cdot 015$ second and the rate of recovery of the nerve would remain unaltered. But it was found that under certain conditions of stimulation the least interval could be increased to a value fifteen or twenty times the absolute refractory period, and about three times the total refractory period. It was thought that these results might be explained by assuming that carbon dioxide has a specific effect similar to that suggested by Jacobs (3) and others in their work on the respiratory centre. But there was also the possibility that the effects were wholly due to the hydrogen ions set free on the ionisation of the carbonic acid formed by the solution of carbon dioxide in the tissue or other fluids. To test this some experiments were also carried out with acetic and hydrochloric acids.

Method. The experiments were carried out on frogs' sciatic gastrocnemius preparations. The muscle-nerve chamber and stimulating and recording arrangements were similar to those used for asphyxiating a nerve, but the nerve chamber for the carbon dioxide experiments had the form shown in Fig. 1, the chamber *B* could be separated by vaseline plugs from *A* and *C*, and a glass plate could be sealed over it; there were also inlet and exit tubes for the carbon dioxide. In *C* there are fluid electrodes for stimulating the nerve central to the region of decrement; these are spoken of as the "outside" electrodes; the other electrodes in *B* are called the "inside" electrodes. For experiments with other acids a similar chamber was used but the "inside" electrodes were of the fluid type and the nerve could be surrounded by a solution of the acid. The

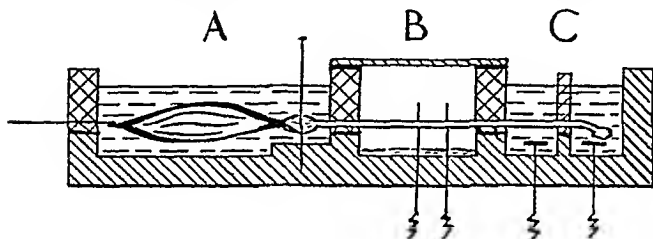


Fig. 1.

chambers *A* and *C* contained Ringer solution. In the gas experiments the preparation was set up and then left with a current of damp air flowing over the nerve until the least interval was steady; the time necessary for this usually varied somewhat with the season. The carbon dioxide was obtained from a cylinder and passed through a wash bottle of water before reaching the nerve; it was also needful to have blotting paper soaked in Ringer solution on the floor of the chamber.

Action of carbon dioxide. When carbon dioxide was passed over the nerve, and the "inside" electrodes only were used, there was an immediate rise of the least interval, a value of less than .015 second was reached in 20 minutes or less, the threshold also fell and the nerve ceased to conduct. The contractions fell off in large steps. If the carbon dioxide was then replaced by air, the nerve recovered at once and in 10 minutes or less it appeared normal again. A typical curve is shown in the inset in Fig. 2.

If the "inside" electrodes were disconnected throughout the experiment, and only the "outside" electrodes used, quite a different result

was obtained. On first passing the carbon dioxide there was a slight fall of the least interval, suggesting a preliminary stimulating action, then the interval began to increase, following the course of the curve obtained with the "inside" electrodes; in Fig. 2 the main curve is obtained using the "outside" electrodes, and is on the same scale as the inset. But with the "outside" electrodes, when the interval had reached a value varying between $\cdot 006$ and $\cdot 025$ second, some other factor seemed to come in; conduction did not fail completely as might be expected, but on the contrary the least interval usually fell slightly and assumed a steady state often lasting two or three hours. Then there was a further rise of the least interval and the remarkable point about this was that it reached a value a good deal larger than the total refractory period (*i.e.* the time taken by a fresh nerve for complete recovery). In some cases it attained a value of $\cdot 05$ second, at which time conduction failed, the whole process having taken three to six hours. If now the carbon dioxide was replaced by air, the least interval dropped almost more rapidly than it could be measured, but it was in the region of the normal value in less than two minutes, and in five to ten minutes the nerve seemed fairly normal again, except that the contraction never quite recovered to its first size. Table 1 gives the figures for some of the experiments. The final values of the least interval are not quite comparable because of the high temperature coefficient, but the last column gives the ratio of the final to the normal value and it shows an average of 20.5. (In Exps. 1 and 2 the final value given is not maximal because a spring contact breaker of small range was used and its limit was reached; in the other experiments a Lucas pendulum of much greater range was used.)

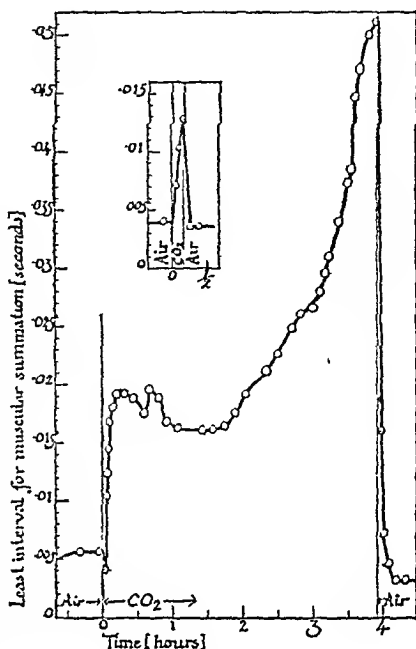


Fig. 2. Action of carbon dioxide. Course of least interval for muscular summation. Main curve: "outside" electrodes only. Inset: "inside" electrodes only (same scale as main curve).

These experiments suggest that stimulating the nerve in the region exposed to carbon dioxide causes a rapid loss of conductivity before

TABLE I

Exp	Temp °C	Normal Least interval secs	Prelim rise maximum secs	Time to prelim rise mins	Steady value of least interval secs	Final value of least interval secs	Time for loss of conductivity h. m	Recovered in mins	Final value Normal value
1	10	0036	013	50	012	> 034	4 0	10	—
2	12	0034	0072	30	0056	> 041	5 5	15	—
3	14	0013	006	20	0047	045	6 0	10	25
4	13	0016	025	40	0185	036	3 33	7	22.5
5	11.6	{ 003 to 005 }	0188	15	0155	053	3 58	12	{ 13 [normal value unsteady]
6	15	0016	0102	20	0063	035	2 48	20	22
7	18	0016	0035	10	003	032	2 15	10	20

any slowing of recovery has taken place. It appears, however, that the loss of conductivity is hastened by stimulation even before the carbon dioxide is applied, for if the "inside" electrodes were used before the passage of carbon dioxide, then on using the "outside" electrodes only, the least interval gave a curve much more like that obtained with the "inside" electrodes alone. If the amount of "inside" stimulation was small, then the least interval sometimes exceeded the value for the total refractory period, but conduction was lost after much shorter time and there was no preliminary rise, recovery was again very rapid. If, during the course of an experiment with the "outside" electrodes, the "inside" ones were used, it was found on using the "outside" ones again that the least interval immediately rose to about 0.15 second and conduction failed, or if this value had been exceeded when the "inside" ones were used, there was no response to subsequent stimulation "outside." The remarkably rapid recovery from carbon dioxide was noticed in every case in which the air was turned on as soon as conduction failed, but an overdose of carbon dioxide quickly killed the nerve.

Adrian (4) found that nerves which had been exposed to acid showed a *supernormal phase* of excitability and conductivity during recovery, in this period a stimulus of less than the normal threshold strength was effective, nerves exposed to alkali showed no such effect. During the course of some of these experiments a series of recovery curves was made, these curves show to a remarkable degree a *supernormal phase*, in spite of the fact that the stimulating electrodes were not on the part of the nerve exposed to the carbon dioxide and the pH of the fluid at the stimulating electrodes was kept either neutral or very slightly alkaline. Figs 3 and 4 show a series of such curves from Exps 5 and 7. Curves I in each case show the nerve before the passage of carbon dioxide and Curves II (the dotted lines) were taken 15 minutes after the carbon

dioxide was turned on and about at the maximum of the preliminary rise of the least interval; they show no supernormal phase. The later curves show the development of the supernormal phase, the percentage excitability rising to 123 p.c. of the resting value in Fig. 4. Just before

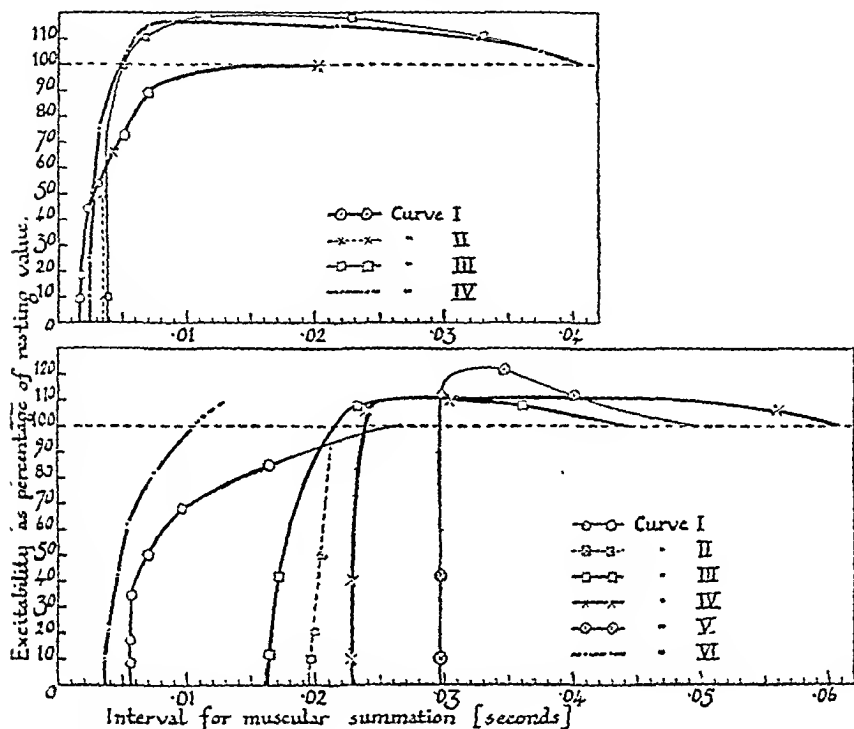


Fig. 3. Recovery curves from Exp. 7. Temp. 18° C.

Curve I. Before CO₂.

II. 15 mins. after CO₂ turned on. No supernormal phase.

III. 1 h. 15 m. after CO₂ turned on. Marked supernormal phase.

IV. 15 mins. after CO₂ off. Marked supernormal phase.

Fig. 4. Recovery curves from Exp. 5. Temp. 11.6° C.

Curve I. Before CO₂.

II. 15 mins. after CO₂ turned on. No supernormal phase.

III. 1 h. 45 m. after CO₂ turned on. Supernormal phase.

IV. 2 h. 30 m. after CO₂ turned on. Marked supernormal phase.

V. 3 h. 10 m. after CO₂ turned on. Marked supernormal phase.

VI. 20 mins. after CO₂ turned off.

conduction failed, it usually happened that there was no response to the first or second stimulus alone, but the two together would give summation at a value which probably indicated the maximum of the supernormal phase. The fact of the two stimuli acting where one alone is ineffective, indicates a phase of increased conductivity. The total duration of this

period when one stimulus is ineffective, but two are effective, is difficult to measure accurately, for in endeavouring not to fatigue the nerve unduly, its onset is often missed. But it has been found to last for as long as 20 minutes, though about 5 minutes is the more usual time. The nerve always showed a supernormal phase when the carbon dioxide was replaced by air, and the phase remained long after complete recovery had taken place. Fig 3 curve shows this very well, but Fig 4 was from an earlier experiment and the final recovery curve (Curve VI) was not completed, it rises quickly and certainly indicates a supernormal phase.

If we are right in supposing that the recovery curve is an expression of the return of excitability in the nerve under the stimulating electrodes (4), the development of a supernormal phase in the curve must mean that a change has taken place in the nerve at the "outside" electrodes although the carbon dioxide is not applied directly to this part. The most likely explanation is that the carbon dioxide or something related to it, has entered the nerve inside the chamber and spread up and down the fibre until it reaches as far as the "outside" electrode region. Further evidence in support of this view will be mentioned later.

Action of acetic acid In order to compare carbon dioxide with another acid, solutions of acetic acid in normal Ringer solution were made up at a pH of 5, 4, and 3.6 and their action was studied on preparations set up in a muscle nerve chamber with double fluid electrodes, the acid was placed in the "inside" chamber and the "outside" electrodes only used. It was found with solutions of pH 5 and 4 that after 4 or 5 hours there was no change in the least interval for muscular summation. It was noticeable in all these experiments that the contractions fell off gradually all the time, the staircase phenomenon was often seen and summation became less easy to detect, in fact the nerve exhibited all the signs of fatigue. It was only when a solution of pH 3.6 was used (1/1000 acetic acid in normal Ringer solution) that the nerve failed to conduct within a reasonable time. Table II shows the main results with

TABLE II

Exp	Temp °C	Normal least interval secs	Final least interval secs	Time for loss of conduc- tivity		Time between and out and measurable recovery		Final value of I I Normal value of L I
				h	m	h	m	
8	15	0026	029	2	18	—	—	11
9	14	0026	036	2	20	1	30	14
10	13	0029	023	2	48	2	0	7.9
11	15	0025	019	1	41	2	10	7.6
12	14.5	0022	021	2	39	—	—	9.5

('Inside' electrodes
used as well)

this strength of acid. The average maximum value for the least interval in the five experiments is $\cdot 025$ second but the final value is only ten times the original value as compared with 20.5 times in the carbon dioxide experiments in Table I.

There is no evidence for an altered rate of recovery. Fig. 5 which is taken from Exp. 9, shows a curve that is remarkable for its extreme smoothness and it was characteristic of all the experiments that there was a perfectly steady period lasting just over an hour, then an unbroken rise until conduction failed. In most of the experiments the summation was very definite, thus making it possible to measure the least interval

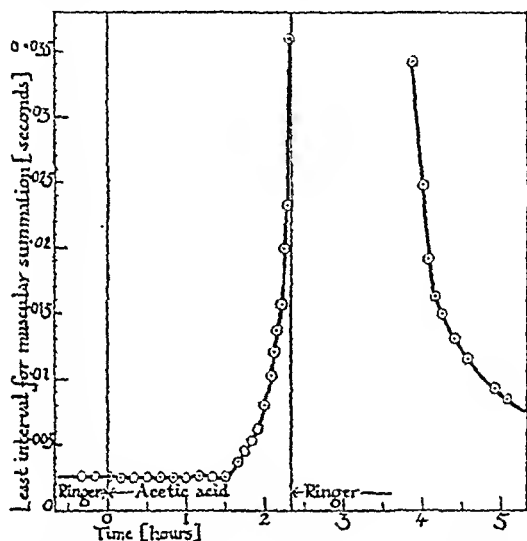


Fig. 5. Action of acetic acid. Course of least interval for muscular summation.

accurately. The contractions remained large for a time, then fell off slightly up to about 10 minutes before complete loss of conductivity, when they fell off in large steps resembling very much the experiments with carbon dioxide when the nerve was stimulated "inside" and the least interval did not increase beyond the limits of the total refractory period. In Exps. 10 and 11 the interval for muscular summation was also measured, using a second stimulus of twice the threshold strength, thus obtaining the "recovery time" as defined by Lucas in his experiments upon alcohol. This interval was found to become slightly less during the course of the experiment, and there was no evidence of it increasing when the least interval rose. The recovery curve gradually developed a supernormal phase; this, as was suggested with the carbon dioxide, probably indicates the entry of the acetic acid into the nerve

fibre and its passage internally to the region of stimulation. It enters fairly slowly and thus does not set up a region of decrement at once as is the case with carbon dioxide. The slow entry of the acid is correlated with its slow exit; this is shown in Table II and also in Fig. 5; a period of one and a half to two hours elapses before the nerve begins to recover, and this it does fairly slowly, in no wise resembling the rapid fall of the least interval after the passage of carbon dioxide.

This probably points to a distinct difference between acetic acid and carbon dioxide, the latter is quick to enter the nerve and equally quick to diffuse out again; acetic acid is more highly ionised and Loeb (5) suggested that for acids to have a physiological effect, they must first be absorbed through the cell wall, and for this absorption the undissociated acid molecules and not the hydrogen ions are responsible. Acids can be arranged in a series according to their power of penetrating living tissues; in this series the mineral acids may be grouped together as being highly ionised and of low penetrative power; with the fatty acids on the other hand, the higher the acid, the less is it ionised and the greater its penetration. Carbonic acid occupies a position at the head of the list as it is very little ionised. Gray (6) has recently confirmed this penetration power of acids in his work on the mechanism of ciliary movement in *Mytilus*, and Hartree and Hill (7) have come to the same conclusions in their recent work on the recovery process of muscle.

Action of hydrochloric acid. Similar experiments were carried out with hydrochloric acid and the results may readily be explained by assuming that the action is much more on the surface than is the case with the other acids. It was found that on using the "outside" electrodes only a solution of hydrochloric acid in Ringer solution giving a pH of 4.2 had no action on the nerve in $4\frac{1}{2}$ hours. A solution of pH 3.5 (1/2000 HCl in Ringer solution), beyond causing a slight fall of the least interval, had no effect until after an hour; it then became difficult to take measurements, probably owing to partial decomposition on the surface. With acetic acid after exposure to a pH of about 3.5 the nerve remained non-conducting for under 2 hours, with hydrochloric acid a considerable longer time was necessary before there were any signs of recovery and complete recovery never took place.

The main points of interest in connection with the present research were the probable absence of any supernormal phase at the "outside" electrodes when the peripheral part of the nerve was in hydrochloric acid, and the limit of the least interval not exceeding .015 second if fresh preparations were used. It is of interest to note that if a preparation

24 hours old is used, the recovery curve shows a supernormal phase when the nerve is in Ringer solution and on the application of hydrochloric acid the limits of the least interval vary, but they tend to exceed .015 second, suggesting that the long least interval depends on acid in the interior of the nerve.

Experiments with carbon dioxide in solution. Some experiments were attempted in which carbon dioxide was passed through Ringer solution and the resulting solution was applied to the nerve; in this way it was possible to obtain a H-ion concentration much nearer the neutral point, and in every case there was a small but appreciable rise of the least interval during the first few minutes. With a pH of about 6 this gave place to a steady period which lasted indefinitely. The experiments had to be abandoned owing to the difficulty of maintaining a constant pH, but they were sufficient to show that the preliminary rise is not merely the result of undiluted carbon dioxide on the nerve. They also suggest that the rise of the least interval and steady period observed in the present research, may have some counterpart in the nervous tissue of the living body. But it is impossible to draw any conclusions in our present state of knowledge, for complications instantly arise when we consider that the nerves probably have to deal with carbon dioxide produced internally as the result of their own activity, as well as with carbon dioxide brought externally in the blood supply.

DISCUSSION.

The whole subject of the action of narcotics and other substances on living membranes has been very adequately reviewed by Lillie (8) in his recent book. The main points to be emphasised here are the work of Overton (9) which shows that the action of narcotics is probably due to their lipoid solvent nature, and the more recent work of Adrian (4) which shows that nerves are very susceptible to any change in the hydrogen ion concentration.

To deal first with the part of the nerve exposed to carbon dioxide. We may suppose that the carbon dioxide is acting here as a narcotic which dissolves very readily in the nerve membrane; if the membrane was otherwise undisturbed it might become saturated with carbon dioxide and allow no more to pass into the nerve. But on stimulation the membrane is altered so that more carbon dioxide is allowed to enter than the interior of the nerve fibre can adequately cope with, and the decrement already set up is increased and fairly soon the nerve ceases to conduct.

But the main point of interest in the foregoing experiment lies in the fact that if the stimuli are sent in at a point outside the region to which the carbon dioxide is applied, the preliminary rise of the least interval is succeeded by a lengthy period in which the nerve seems to have reached a new equilibrium with its surroundings. The attainment of this equilibrium is associated with the development of the supernormal phase in the recovery curve. Figs. 3 and 4 show that the rate of recovery is unaltered during the preliminary rise and they show the development of the supernormal phase as the steady period succeeds the rise. The supernormal phase appearing at the outside electrodes has been explained as due to a diffusion of product from the affected area in the interior of the fibre as far as the "outside" electrodes, and if this is so it evidently implies a change in the interior of the fibre rather than a surface effect. The most likely change would be an increased acidity due to the ionisation of the carbon dioxide which has entered the fibre, but in any case the steady period seems to express some sort of balance between the external carbon dioxide acting as a narcotic and an internal effect which counteracts this and is associated with the "supernormal" type of recovery curve. If the carbon dioxide was diluted with some other gas, this balance might be maintained indefinitely as was the case with the carbon dioxide dissolved in Ringer solution; it has been noticed that a certain concentration of all narcotics or other substances that cause a decrement has to be reached before there is very much effect. In the present case the balance is not maintained indefinitely and eventually the interval for summation rises again and conduction fails. This may perhaps indicate that the capacity of the nerve for absorbing carbon dioxide is limited and it is a significant fact that if the carbon dioxide is left on for a few minutes after conduction has completely failed, there is no recovery, whereas recovery is always rapid and complete if conduction fails in the early stages before the steady period develops.

It is difficult to make certain whether the carbon dioxide does cause a slowing of the rate of recovery of excitability and conductivity in the nerve. The interval for muscular summation certainly rises much above the value which is generally taken for the total recovery time of the normal fibre, *i.e.* .015 second, but this time is given by the moment at which the excitability first returns to its normal resting value. If this is followed by the supernormal phase it is clear that the nerve has not really come back to its resting condition until this phase is over, and the long least intervals obtained with carbon dioxide are not outside the range of the complete recovery time. This is not always easy to measure, but

in Fig. 3 it has a value of about $\cdot 04$ second, and the final least interval was between $\cdot 03$ and $\cdot 04$ second; in Fig. 4 the complete recovery time is about $\cdot 06$ second and the final least interval was over $\cdot 05$ second.

Other acids have a much lower penetrative power; they do not get inside fast enough to cause the preliminary decrement or the rapid development of a supernormal phase. The high final value of the least interval seems to be associated with the supernormal phase, for if a frog's muscle nerve preparation is kept in Ringer solution for some hours, the recovery curve shows a considerable supernormal phase and treatment with acetic or even hydrochloric acid tends to give a high final value of the least interval. Possibly the condition of the frog also plays some part in the process.

With regard to the effect of acids in general it seems necessary for the hydrogen to get into the nerve in an unionised condition, this makes the acid radicle important; the penetrating power of acid is probably controlled by the lipid nature of the nerve membrane, and the acid radicle thus becomes a "carrier" for the hydrogen. Carbon dioxide is specific in so far as it dissolves very readily in the lipoids of the membrane and is thus able to penetrate to the interior of the fibre very rapidly. Its powers of penetration are comparable with those of other narcotics, and hence it shows a narcotising action before the effect of the hydrogen ion is seen.

SUMMARY.

Experiments have been carried out to find the effect of carbon dioxide on the excitability and conductivity of a frog's sciatic nerve. Other experiments with acetic and hydrochloric acids were performed in order to compare the results with those obtained with carbon dioxide.

1. If the nerve is stimulated on the region exposed to carbon dioxide, it immediately begins to conduct with a decrement, conduction fails in about 20 minutes when the least interval has reached a value of about $\cdot 015$ second, there is no evidence for an altered rate of recovery.

2. If the nerve is stimulated with fluid electrodes on the part central to the carbon dioxide, then there is a preliminary rise of least interval, this reaches a maximum, there is a slight fall and a steady period lasting over an hour, then a final rise. Conduction fails in about 4 hours and the least interval reaches an average value of $\cdot 04$ second or about twenty times the normal least interval. The least interval falls as soon as the carbon dioxide is replaced by air. A marked supernormal phase is developed during the course of the experiments.

3 In similar experiments with acetic acid in Ringer solution at a pH of 3.6 and central stimulation, there is no preliminary rise and conduction fails in about 0.25 second, or ten times the normal least interval, this is comparable with the total refractory period and the rate of recovery is unaltered. A supernormal phase is developed, but much more slowly than with carbon dioxide. A period of nearly 2 hours elapses before there is any recovery when acid is replaced by Ringer solution.

4 With hydrochloric acid the least interval is much more difficult to measure, but it probably does not exceed 0.15 second with a pH of 3.5. Recovery is very slow and rarely complete.

5 Carbon dioxide probably acts first as a narcotic, and, if the nerve is stimulated in the narcotised area, only as a narcotic. But if it is stimulated central to this area, the carbon dioxide enters the fibres and an equilibrium is set up between the external carbon dioxide acting as a narcotic and an internal effect which is associated with the "supernormal" type of recovery curve. This equilibrium is not maintained and finally the least interval rises and conduction is lost. Owing to the supernormal phase, the time for complete recovery has been lengthened and this is associated with the high value of the least interval.

I wish to acknowledge my thanks to Dr Adrian for his helpful advice throughout the work.

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A CIRCULATING SCHEME FOR RECORDING RESPIRATORY METABOLISM. By E. H. J. SCHUSTER.

(From the National Institute for Medical Research, London.)

THE apparatus was originally designed and made at the suggestion of Dr Lovatt Evans, and in its original form was intended to record the oxygen consumption of an anaesthetised animal breathing naturally. It has been modified for the work of Drs Burn and Dale, who were using the decapitated preparation, by inclusion of the double-action respiration pump, previously described by me (*J. Physiol.* 56, Proc. Physiol. Soc. p. x. 1922), and in that form is here figured. For use with the anaesthetised animal, breathing naturally, the pump need merely be omitted, and a single pair of valves substituted for the double valve-system shown in the diagram.

Fig. 1 shows a general plan of the apparatus in a purely diagrammatic manner. P_1 and P_2 are the two barrels of the double-action respiration pump. They are connected respectively with the valves (V_1 and V_2). The suction nozzle of the valve (V_1) is joined by rubber tubing to the outlet of the spirometer (Sp) and its delivery nozzle to a tube tied into the trachea of the spinal preparation. The pump barrel (P_1) therefore withdraws air from the spirometer and delivers it to the animal. The suction and delivery nozzles of the valve (V_2) are connected respectively with the animal, and, through a soda-lime tower (SL) with the inlet of the spirometer, so that the barrel (P_2) draws air from the animal and delivers it through the soda-lime to the spirometer. If it is desired to measure the output of carbon dioxide, a train of weighed absorption tubes is substituted for the soda-lime tower. The pump pistons work in unison so that the

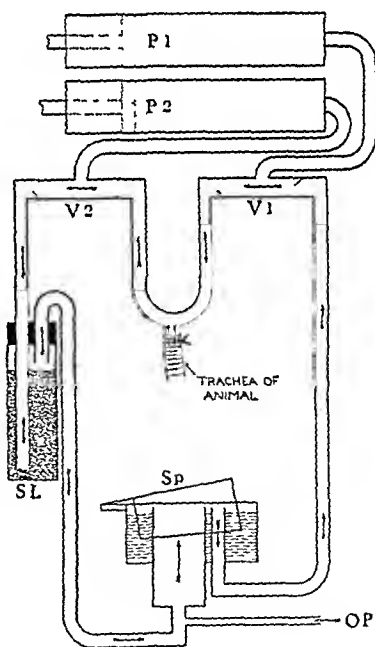


Fig. 1.

rise and fall of the spirometer cap synchronise with the expiration and inspiration of the animal. A side tube (*OP*) from the spirometer inlet is connected with the oxygen pump.

Fig. 2 shows the spirometer, the cap and tank being depicted as if transparent. The cap (*Sp.C*) is carried on brackets which are pivoted on centres at *C*. The brackets are shaped to clear the side of the tank.

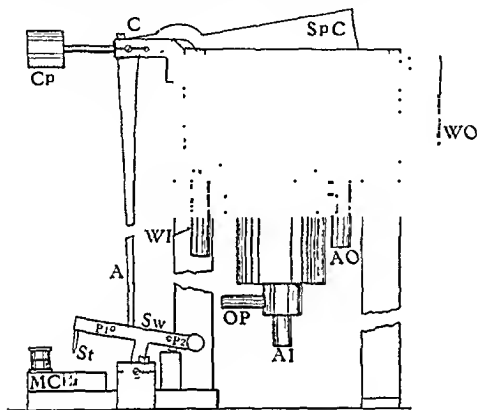


Fig. 2.

Attached rigidly to them is the counterpoise weight *Cp* and the long, light arm *A*.

The spirometer cap rises and falls as air is pumped into it and withdrawn from it, but the volume of air in circulation tends always to diminish because oxygen is being used up by the animal and the carbon dioxide produced is absorbed in the soda-lime. Therefore, unless the loss is made good, the spirometer cap at each stroke of the respiration pump rises less high than on the previous stroke and falls to a lower level. The arm (*A*) swings to the right and to the left as the cap rises and falls, the end of each swing tending to move more and more to the left. When the cap sinks below a certain point, the arm (*A*) presses against the pin (*P*₁) carried on the switch (*Sw*). This knocks over the switch from the no-contact position shown in the figure to contact position. Contact is made by two steel points (*St*) entering two mercury cups (*MC*). Only one can be seen in the view drawn as the other is immediately behind it.

At the top of the next stroke the arm engages with the pin (*P*₂) and

suction stroke is in the position shown at *b*, is forced into the position shown at *a* because, owing to the friction in the gland, the pump plunger does not move relatively to the sleeve until it has forced the sleeve as far as possible in the direction along which it is travelling. When this position is reached, a port in the sleeve registers with a port in the bore of the pump connected with the delivery nozzle, and the further forward

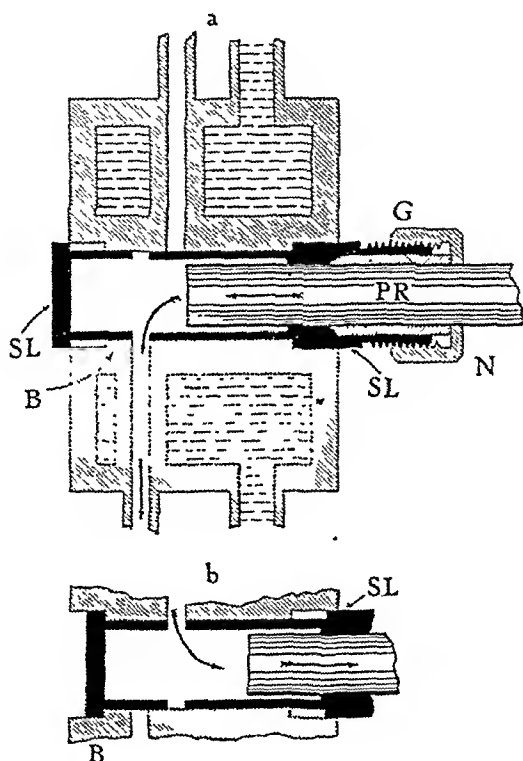


Fig. 4.

movement of the plunger drives a definite volume of oxygen out of the sleeve. The reverse of these operations happens on the suction stroke: first the sleeve is drawn back to the position shown at *b* in which the suction ports are in register, and during the remainder of the stroke the same volume of oxygen as was previously expelled is drawn into the sleeve.

In order to ensure that the pump shall be maintained at the same temperature as the spirometer it is waterjacketed, and water is circulated first through it and then through the spirometer tank, entering the latter through the nozzle *WI* (Fig. 2) and leaving by the overflow nozzle (*W.O*).

THE EFFECT OF CEREBRAL ANÆMIA UPON BLOOD-PRESSURE AND RESPIRATION.

By FR. ROBERTS.

(From the Physiological Laboratory, Cambridge.)

IN a previous communication (*This Journ.* 55. p. 346, 1921) it was shown incidentally and in confirmation of earlier workers that in the anæsthetised animal the sudden stoppage of the blood-supply to the brain by clamping the four arteries or by rapid bleeding from the abdominal aorta resulted in an immediate stoppage of respiration; the main effects of acute anæmia upon the respiratory centre was paralysis. In this paper are presented in more detail the effects of anæmia upon the medullary functions. Although a great amount of work has already been performed upon this subject it has been concerned almost entirely with the results immediate and remote of complete occlusion of the four main arteries. Even those such as Hürthle who have studied the effects of varying degrees of anæmia have confined their attention to the changes in blood-pressure and have neglected to enquire how far such changes as they observed were due to the action of the vaso-motor centre and how far to the action of the cardiac centre.

The principle followed in the experiments here described has been to subject the brain to a diminishing blood-supply by clamping the arteries supplying it, a record being taken where possible of the pressure of blood in the Circle of Willis, that is to say the pressure at which blood passes to the cerebral arterioles. The experiments were all performed upon rabbits and cats with exception of two upon dogs. The cats and rabbits were anæsthetised with urethane (1 gm. per kgm. body-weight), and some very lightly with C.E. mixture. For the dogs morphia was used. The experiments as performed upon the rabbit were as follows. A tracheotomy tube having been inserted, the subclavian artery was dissected out on each side so as to expose the origin of its branches. Of these the vertebral is the most proximal. The main artery was ligatured at a point just distal to its branches and a loose ligature passed round the artery proximal to the origin of the vertebral. In these pages the expression "clamping the subclavian" means clamping it at this point, a procedure which blocks all the branches without causing any indirect effects from the sudden stoppage of blood to the upper limb since the

main blood-supply to this part of the body has been permanently closed. The common carotid is then dissected out on each side and the thyroid and external carotid branches ligatured. Into one of the common carotids two cannulae are inserted, one pointing downwards in the usual way and used to record the aortic pressure, the other pointing upwards into the brain. The tube connecting this one with the mercury manometer is filled with normal saline since magnesium sulphate and the citrates are not without effect upon the vaso-motor and respiratory centres and would of course reach the medulla from the manometer, whenever the cerebral pressure were to fall. The other common carotid is prepared for subsequent compression by passing a loose ligature round it. For clamping the arteries small bull-dog clamps are employed of a strength just sufficient to ensure complete occlusion without causing damage to the vessels with subsequent thrombosis.

For recording respiration two methods were adopted. The one more generally adopted was to connect one limb of the Y-shaped tracheotomy tube with a tambour of the thinnest rubber. This method has the merit of giving a very sensitive though not standardised record of the rate and depth of respiration but it has the disadvantage of not recording the actual respiratory movements or the state of the chest at any given moment. When it was desired to record these the movement of the abdominal wall was registered by a lever connected to the abdominal wall.

The two essentials in experiments of this sort are that the method of registration should be sufficiently sensitive to record the smallest changes and that the medullary centres should not be depressed by the anaesthetic or by the preceding operative procedures. That these conditions were fulfilled was shown by the fact that when the animal was made to re-breathe its own expired air there was recorded after 30 seconds a well-marked and after 60 seconds a violent dyspnoea. That the centres were in a healthy condition was further shown by the good blood-pressure which was always obtained and by the presence of the usual reflexes characteristic of light anaesthesia.

The upper blood-pressure record gives, I believe, fairly accurately the existing pressure in the Circle of Willis except possibly when this pressure is very low. It responds readily to changes in the aortic pressure. In amount it averages, as Hürthle who was the first to use this method found, about two-thirds of the aortic pressure but it may as in Fig. 3 be much more. This is of course less than the normal since one artery is already tied. What the normal Circle pressure is, is not known. It cannot be determined, as Hürthle states, by taking a record from the

ophthalmic artery since this artery itself contributes blood to the Circle. It is probably not far below the aortic pressure.

The method of experimentation here adopted has several merits including those of simplicity and of rapidity in preparation, the animal being in a good state when the observations are being made. But the greatest merit is the unmistakeable origin of any phenomena observed. When the blood-supply is altered this is done in such a way that only the brain is involved for the blood-supply to the rest of the body is not interfered with except for the very small rise of pressure passively produced. The first changes in circulation and respiration must therefore be the direct results of ischæmia or anæmia of the brain and cannot be due to anything else. Later effects will of course have a more complicated causation. If, for instance, respiration is stopped by anæmia for some time, the effects which follow the re-admission of blood to the brain will be referable partly to the altered condition of the respiratory centre and partly to the altered condition of the blood. Such effects must therefore be interpreted with caution.

I believe that this method of experiment is superior for my purposes to the method of crossed-circulation. In the latter method there is a serious fallacy especially when it is applied to the dog. In the dog occlusion of the four main arteries to the brain does not lead to asphyxia owing, as L. Hill has pointed out, to the very free anastomosis which exists by way of the intercostal and anterior spinal arteries. The brain of the "fed" animal is therefore not exclusively supplied by the "feeder" but by both animals. It follows therefore that when the blood from the "feeder" is reduced the "fed" dog receives a proportionally larger amount of its own blood which will be at a different H⁺ ion concentration from that of the "feeder." To a lesser extent this objection applies also to the cat and rabbit for as will be seen later in these animals although occlusion of the four arteries produces violent disturbances of medullary function there is often a tendency to recover even while the arteries remain closed. In the rabbit too the quadruple ligature does not annul the Circle pressure.

I. *The circulatory effect.*

Clamping the carotid on the side not already blocked. When the opposite carotid is clamped there is in general a rise in aortic pressure. But this is not constant as Gaskell and Shore⁽¹⁾ state and in different animals it is very variable in amount being anything up to about 30 mm. (see Fig. 2). Sometimes in the cat there is a fall in pressure. The rise when it occurs is doubtless due partly to a passive

effect but not entirely. This is shown by the fact that the rise exceeds that which is produced on clamping an artery of equal size which does

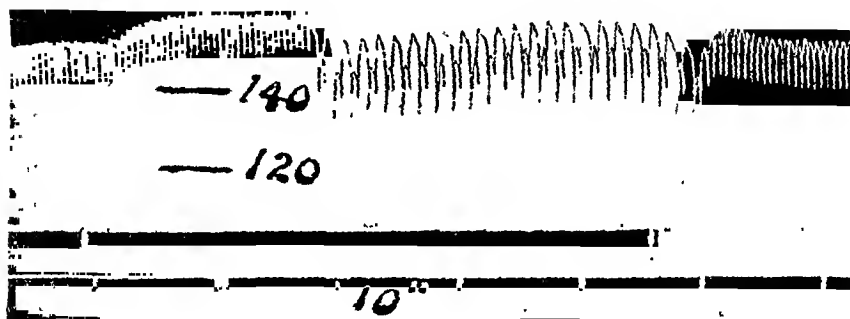


Fig. 1. Cat. Blood-pressure from femoral artery. Between the signals one of the carotids was clamped.

not supply the brain and also by the effect recorded below of cutting the vagi. Part and probably the greater part is to be attributed to the well-known sensitiveness of the vaso-motor centre to its own blood-supply. Whether this response is to the pressure or to the quantity of blood is a question not yet answered. What is the cause of the individual differences found? Those who have studied the reaction of blood-pressure to changes in posture have ascribed the individual differences to differences in the sensitiveness of the vaso-motor centre. The degree of anaesthesia however light no doubt is also partly responsible. But there is another factor which has never been taken into account and that is the concurrent effect upon the cardio-inhibitory centre. In the cat when a fall occurs there is obvious slowing of the heart (Fig. 1) and in the

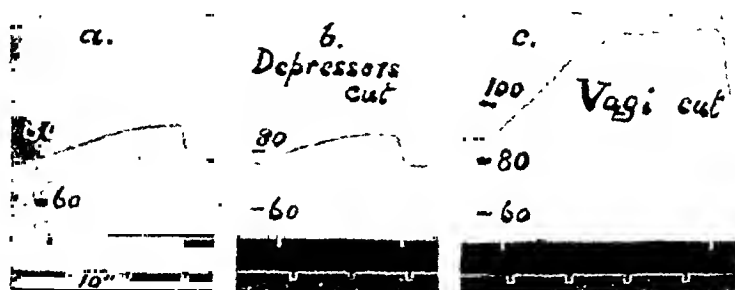


Fig. 2. Rabbit. Between the signals the carotid was clamped.

rise which usually occurs there is also some slowing. Thus slight anæmia sets in action the cardio-inhibitory as well as the vaso-motor centre and the differences shown by different animals may be attributed to the different sensitiveness of these two centres. The considerable effect of the cardio-inhibitory centre in reducing the rise is shown by cutting the vagi and then blocking the remaining carotid. There is then a much greater rise in blood-pressure and it is more uniform in different animals. The fact that in the cat there may be a primary fall of blood-pressure shows that the cardio-inhibitory action is not due to afferent impulses but is a direct action of anæmia upon the centre.

In the rabbit the results are the same except that with intact vagi there is no slowing of the heart. The vagi do however reduce the rise for when they are cut the rise as in the cat is much greater and more uniform in different animals. This reducing action is not due, or only to a small extent, to the depressors, for the rise is not increased after section of these nerves (cf. Fig. 2). It must therefore be due either to afferent depressor fibres in the vagus trunk or to direct stimulation of the cardio-inhibitory centre by the anæmia causing weakening but not slowing of the heart. From the analogy of the results in the cat and from the improbability of afferent fibres being stimulated I consider that the effect is direct. The individual differences which are found must therefore be ascribed chiefly to the varying degree in which the vagus centre is brought into play in different animals. It is well known that the vagus centre is powerfully stimulated when in a state of extreme anæmia or asphyxia but the fact that a slight diminution in blood-supply stimulates both vaso-motor and cardio-inhibitory centres simultaneously is one which if already known is not generally recognised.

This result leads to an interesting corollary. The sensitiveness of the vaso-motor centre to its blood-supply is generally regarded as the chief factor in the compensation of blood-pressure against disturbances due to gravity. The fact that this sensitiveness is also shared by the vagus centre suggests that an animal would compensate better with vagi cut than with vagi intact.

The effect of clamping the opposite carotid upon the pressure in the Circle of Willis has been studied by Corin⁽²⁾, Hürthle⁽³⁾, and Gaskell and Shore with inconsistent results. Gaskell and Shore working on the dog found a fall in pressure with no tendency to recover. Hürthle found a fall followed by partial recovery of variable extent. Corin found a fall only of momentary duration followed by a rise sometimes actually to a higher level than that which obtained before the occlusion.

This result is doubted by L. Hill(4) who believes that it could only occur if the original pressure were very low. Hürthle is also sceptical and rightly comments on the inadequate information given by Corin regarding the aortic pressure. In my experience closure of the carotid causes a lowering of Circle pressure which remains at the lower level if the aortic pressure does not rise. But if the aortic pressure does rise the Circle pressure tends to recover slightly and in some cases up to its original level. Rarely however Corin's paradoxical result occurs. Clamping the carotid while producing a rise in aortic pressure causes a rise in Circle pressure to a height well above its previous level, the high pressure remaining until the artery is released (Fig. 3). It is difficult to

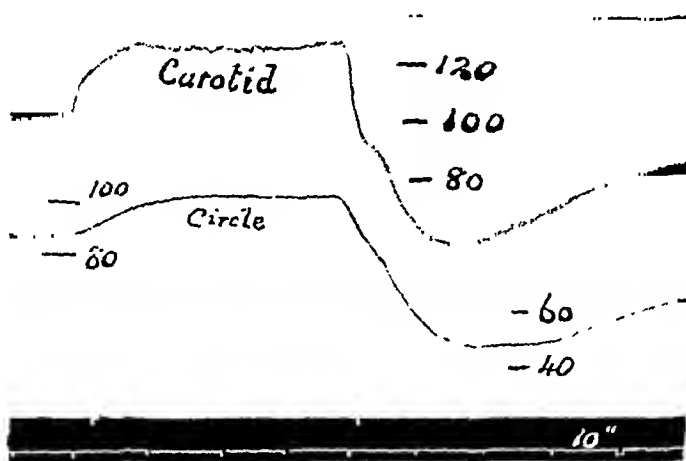


Fig. 3. Rabbit. Between the signals the carotid was clamped. This tracing has been touched up for the purpose of photographic reproduction but the general contour has not been altered.

give an adequate explanation of this phenomenon but it may be that in these cases the supply of blood by the vertebrals was unusually great and that the rise in aortic pressure increases the pressure in the Circle. A very free vertebral contribution is certainly indicated by the remarkably high Circle pressure which obtains in these cases before any of the arteries are closed.

Clamping of one of the subclavians tends to produce in comparison with clamping of the opposite carotid an equivalent fall in Circle pressure but a smaller rise in aortic pressure. This fact also appears from an analysis of Hürthle's figure but he makes no comment on it.

Severe anæmia. In the rabbit with two carotids already clamped, closure of one of the subclavians produces usually a fall in Circle pressure

and a further slight rise in aortic pressure. If now the subclavian of the other side be also closed there occurs a sudden and well-marked rise in aortic pressure together with a profound fall in Circle pressure. A typical example is seen in Fig. 4. The aortic pressure commonly reaches a height of over 180 mm. Vagal inhibition then sets in with well-marked slowing of the heart. This causes a steady fall in pressure until the heart fails, the same picture in fact that is seen in ordinary asphyxia. In those cases in which the pressure was already high, clamping of the fourth artery causes very little further rise for vagal inhibition sets in at once. General muscular spasms and convulsive movements are liable to occur as previous workers on this subject have described but they are by no means constant.

The Circle pressure does not fall to zero. There is, in agreement with the results obtained by Hürthle and by Gaskell and Shore, a residual pressure of between 18 and 6 mm. There is therefore still some blood flowing into the medulla under these circumstances as Gaskell and Shore demonstrated by subsequent injection. Moreover there is evidence which will be shown below that medullary function sometimes recovers though in a modified form while the arteries are still clamped. On the other hand the tendency to recover bears no relation to the height of the residual Circle pressure and such collateral circulation as must persist does not influence the manometric reading of the Circle pressure for the tracing does not show any change even with the most violent changes in aortic pressure.

It is stated by L. Hill (5) that the bulbar centres are first excited and then paralysed but that when the anæmia is slow in onset the excitatory symptoms fail to appear. I have put this to the test by subjecting the brain to a very gradual reduction in its blood-supply by closing the carotid very slowly with a screw-clamp, the other arteries having been closed. When this is done the great rise in pressure which occurs on

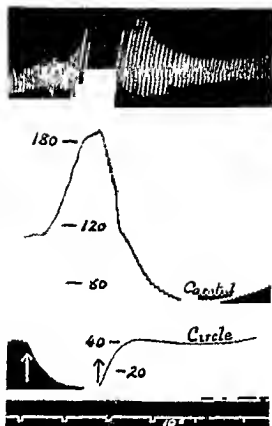


Fig. 4. Rabbit. At the first arrow the R. subclavian was clamped, the other arteries being already closed. At the second arrow the carotid was opened.

sudden clamping is absent but this is due not as Hill thinks to an absence of stimulation of the vaso-motor centre but to the fact that vagal stimulation by the anæmia sets in earlier. After section of both vagi the rise in pressure is just as great whether the fourth artery be clamped suddenly or gradually. The effect of gradual clamping with the vagi intact is shown in Fig. 5. There is therefore no doubt that extreme

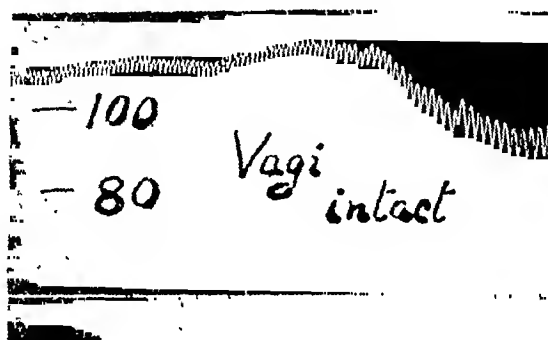


Fig. 5. Cat. This shows the terminal part of gradual clamping of the fourth artery begun three minutes earlier when the pressure was 92 mm.

anæmia is a strong and direct stimulus both to the vaso-motor and to the cardio-inhibitory centre. The rise in pressure when the vagi are cut is not however proportionate to the degree of anæmia. There is a critical Circle pressure of between 18 and 6 mm. at which reduction in blood changes from being a slight to a powerful stimulus.

Recovery. Recovery in blood-pressure is always immediate. When blood is restored after a short occlusion, that is while the pressure is still high, there is a sudden fall in pressure to a subnormal level followed by rise to normal (Fig. 3). When the occlusion has lasted until the pressure is subnormal owing to cardio-inhibition, provided that there is no heart-failure, restoration of blood causes an immediate rise of pressure to the normal partly through the termination of cardio-inhibition (Fig. 10). As a rule the vaso-motor and cardio-inhibitory centres recover simultaneously.

The part played by the suprarenals has not been investigated but the complete absence of any after-effects or delay in the restoration of blood-pressure points to the fact that a possible liberation of adrenalin due to the cerebral anæmia plays very little, if any, part in the rise of pressure.

II. *The respiratory effect.*

Hæmorrhage. Before dealing with the main part of this section a word is necessary on this subject. It is stated by Gesell⁽⁶⁾ that

pulmonary ventilation varies inversely as the blood-pressure. Mainly upon this statement he builds a new theory of the regulation of respiration according to which the metabolism of the respiratory centre is a factor to be taken into account under normal as well as under abnormal conditions. The centre according to this view is influenced not only by the blood reaching it but by the products of its own metabolism. These products are two acids, CO_2 and a fixed acid which is probably lactic. The increased respiration which is said to follow anoxæmia is brought about by diminished oxidation leading to increased acid formation there, such acid through delay in its removal playing a part in the stimulation of the centre. On this view the effective stimulus to the centre would appear to be the actual acidity or hydrogen ion concentration of the centre which is the sum of the acidity of the arterial blood plus the acidity contributed by the metabolism of the centre. When the amount of blood supplying the centre is diminished the removal of products of metabolism from the centre is defective with the result that the centre is stimulated to greater activity. Gesell's main evidence for his theory is the fact recorded by Gesell, Capp and Foote (7) that pulmonary ventilation varies inversely as the blood-pressure. The actual observation upon which this assertion is based reads as follows. "The effect of hæmorrhage on pulmonary ventilation... varied considerably. In some instances hæmorrhage had little or no effect on the pulmonary ventilation of room air and in other instances small hæmorrhages markedly increased the ventilation." And again, "Hæmorrhage and injection (of gum-saline) which elicited the usual respiratory responses not infrequently were accompanied by no or small alterations in the mean blood-pressure."

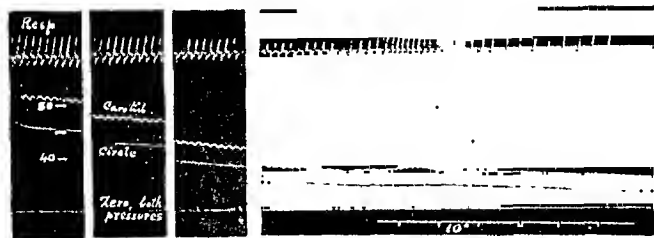


Fig. 6. Rabbit. Hæmorrhage from the abdominal aorta. Between the first and second and between the second and third are intervals of $\frac{1}{2}$ min.; between the third and fourth, 1 minute.

In view of the far-reaching conclusions which Gesell draws and the dubious nature of the experimental evidence upon which they are based I have thought fit to re-investigate the effects of hæmorrhage on respiration. Rabbits and cats were bled into a compensating tube tied into the lower part of the abdominal aorta. Fig. 6 shows the typical result obtained in the rabbit, the hæmorrhage in this case lasting three minutes. It will be seen that so far from there being an increase in respiration there was a decrease both in rate and depth except for a slight transient hyperpnœa which preceded complete apnœa. The hyperpnœa occurred at an aortic pressure of 32 mm. and a Circle pressure of 18 mm. In the cat (Fig. 7) a similar state of things is observed except that the stage of

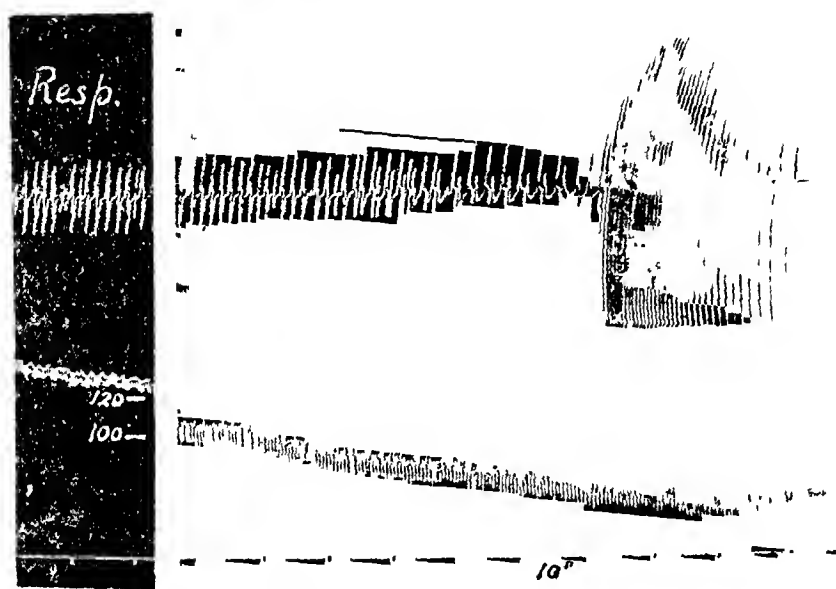


Fig. 7. Cat. Hæmorrhage from the abdominal aorta. Between the sections an interval of one minute.

hyperpnœa is more marked. It occurs however only when the pressure has reached a very low level. On restoring the blood (Fig. 8) respiration is resumed in a staircase manner. From such experiments as these the changes produced by the hæmorrhage are so complex and wide-spread that it would be unjustifiable to refer any respiratory effects to the direct effect of a diminished blood-supply upon the centre. But the fact brought out that pulmonary ventilation does not vary inversely with blood-pressure completely negatives Gesell's assertion and shows that his theory is without experimental foundation.

Occlusion of the vessels. When the respiratory effects are investigated the fact which stands out most prominently is that the respiratory centre

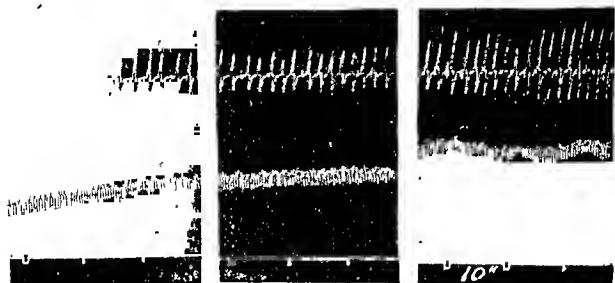


Fig. 8. Cat. Recovery by return of blood. Between the sections intervals of $\frac{1}{4}$ minute.

can withstand, without in the least modifying its action, a very considerable reduction in its blood-supply. When as many as three out of the four arteries are clamped there is no change of any kind in the rate

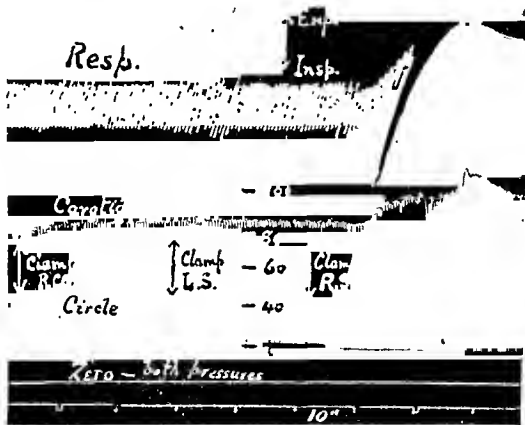


Fig. 9. Rabbit. Description in text.

R.C.C., right common carotid. *L.S.* and *R.S.*, left and right subclavians respectively.

or depth of respiration, whether the artery which remains open is one of the carotids or one of the subclavians. Such reduction in blood-supply brings the Circle pressure down to about 22 mm. as for instance in the typical example shown in Fig. 9. The original pressure of 40 mm. in this case is of course less than the normal Circle pressure since the left carotid had been tied at the beginning of the experiment. The results of a large number of experiments show that the pressure of blood supplying the brain can be reduced to less than a quarter of its normal amount before any disturbance of respiration is apparent.

When however the fourth artery is clamped respiration immediately ceases. The stoppage may be absolutely abrupt (Fig. 4), or the respiratory movements may undergo a rapid reduction in depth with or without reduction in rate. In other cases the respiratory movements may at the

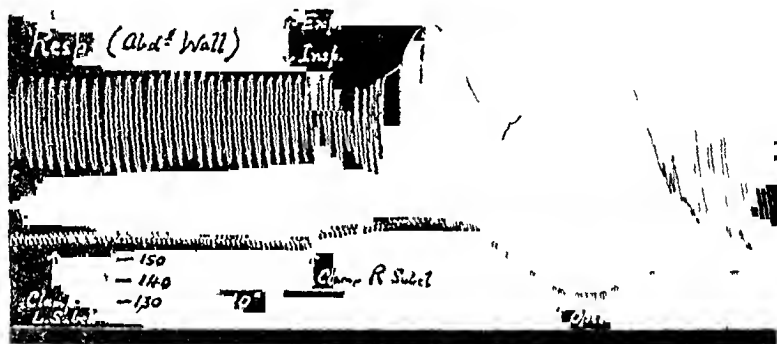


Fig. 10. Cat. Movement of abdominal wall recorded.

last undergo an increase in depth sometimes with diminished frequency but such increase is never more than transitory and never lasts more than six or seven respiratory beats. There is never a well-marked and prolonged period of hyperpnœa, such as would be expected to occur were the respiratory centre as it runs short of oxygen being stimulated by products of its own incomplete metabolism.

To judge from the pressure in the Circle of Willis there is a very narrow margin between the least amount of blood sufficient to maintain normal respiration and the greatest amount which is still insufficient to prevent complete paralysis. Fig. 9 for instance shows respiration continuing normally at a Circle pressure of 22 mm. but completely stopped at a pressure of 18 mm. There is therefore as for the vaso-motor centre a critical pressure below which respiration is violently disturbed. The actual pressure varies slightly in different animals but in any individual

the pressure which is critical for the vaso-motor centre is critical also for the respiratory centre. The effects upon these two centres are simultaneous but opposite, respiration ceasing but blood-pressure rising at the same moment. In Fig. 9 the rise in pressure was counteracted to a considerable extent by cardio-inhibition.

It may of course be said that both centres are in reality affected in the same way, there being a tonic contraction of peripheral muscles in both cases, that is to say the respiratory muscles and the arterioles. Hill for instance speaks of respiratory spasm and vaso-motor spasm. What usually happens is shown in Fig. 10 in which the actual movements of respiration are recorded. Respiration comes to a stop in full expiration. Whether or no this can be described as respiratory spasm the physiological effect is that pulmonary ventilation is completely inhibited.

A word is necessary concerning the hyperpnœa which as we have seen sometimes precedes the apnœa. Since it tends to occur just as the medulla is running short of blood it might be thought to be due to stimulation of the respiratory centre by the acid products of incomplete metabolism. Were this the cause the hyperpnœa should be more marked the more gradually the anæmia is brought about. But the reverse is the case at any rate in the rabbit.

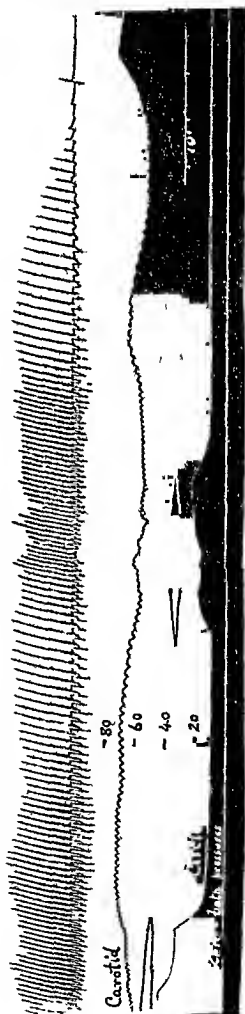


Fig. 11. Rabbit Description in text

Fig. 11 shows the result obtained when the fourth artery is closed by a screw-clamp very gradually. Narrowing of the clamp is shown by the mark > and widening by the mark <, the direction of the change being also indicated by the record of Circle pressure. It will be seen that although there may be very slight increase in depth as the blood-supply diminishes the main effect is a well-marked reduction in rate with restoration of rate as blood is re-admitted. The rate of respiration that is to say can be controlled by varying the blood-supply. In other words the respiratory centre is active only inasmuch as it is supplied with a sufficiency of blood. Sudden anæmia therefore produces conditions which gradual anæmia does not. One is reminded here of the effect of sending a current of gradually increasing intensity into a nerve.

Recovery. If the blood has been shut off only for a few seconds then on re-admitting blood respiration re-starts usually abruptly with hyperpnoea which is due probably to the increased hydrogen ion concentration which the blood has undergone during the apnoea. If on the other hand the anæmia has been allowed to continue the apnoea continues while the blood-pressure falls from vagal inhibition and in this way the animal dies with or without the terminal gasps characteristic of asphyxia. More frequently however respiration re-commences even while the arteries are still clamped. This occurs about half a minute after the beginning of complete occlusion and while the blood-pressure is still high. Each respiratory movement consists of a sudden deep inspiration followed directly by expiration and afterwards a pause. One must assume that in these cases there is established a degree of collateral circulation sufficient to allow the respiratory rhythm to be partially restored and at the same time to prevent excessive stimulation from lowering the blood-pressure. In some instances this state of things would appear to be capable of continuing if not indefinitely at any rate for a long time. In others the gradual fall in pressure and the ever-widening intervals between the respiratory movements lead to death. It may be remarked that in successive occlusions and releasing of the fourth artery the respiration starts more and more readily indicating an increasing collateral circulation.

When to a medulla in this state of modified activity blood is re-admitted blood-pressure as a rule immediately falls to normal. Respiration is restored to normal at the same time or shortly after. Of the way in which it is restored I have already given a brief account⁽¹⁴⁾. In some cases there is merely a rapid quickening up to the normal rate. Very frequently recovery takes place as shown in Fig. 12. On opening an

artery there is first a momentary suppression of the previous rhythm as though the first effect of increased blood-supply were an inhibition.

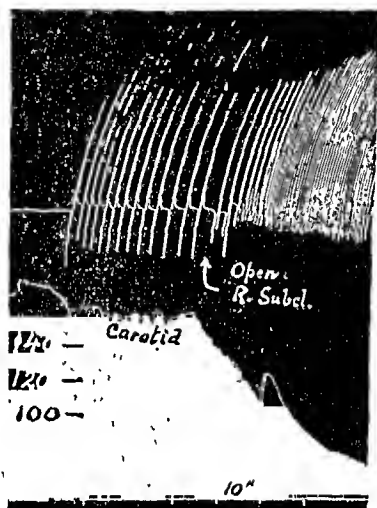


Fig 12. Rabbit. Description in text.

Then respiration is resumed at a quicker rate. After a couple of breaths there appears an interpolated beat which, beginning as a very weak beat, progressively increases in strength up to that of the original beat. In one case I have noted the sudden suppression of the interpolated beat on again clamping the fourth artery. Sometimes the beat may be approximately doubled in rate suddenly as occurred in the case shown in Fig. 13 when the left vertebral was opened. The slight improvement in the blood-supply which this procedure caused is shown on the tracing of Circle pressure. On subsequent opening of the left carotid an interpolated rhythm set in so that in effect the original beat was first about doubled then about quadrupled. These phenomena appear to me to indicate that the central mechanism of respiration resembles the heart-beat in that it consists of two processes, the initiation and the conduction of a disturbance. In recovery from anæmia we observe in those cases where the rate is progressively quickened a recovery in initiation with normal

conduction and in those cases in which an interpolated rhythm appears gradual recovery in conduction.

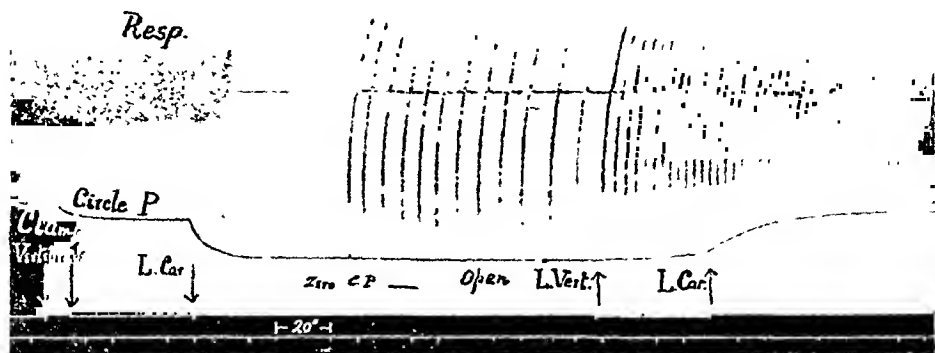


Fig. 13. Rabbit. Description in text.

Lumsden(s) in a recent series of papers has expressed the view that there are three centres concerned in respiration; the lowest or "gasp" centre; above this an "apneustic centre," which when uncontrolled causes respiration to take the form of prolonged inspirations; above this again, in the upper part of the pons, a "pneumotaxic" centre which produces normal respiration by periodic inhibition of the apneustic centre. The effects of occlusion in the cat are according to Lumsden as follows: "About half a minute after the blood is completely shut off the pneumotaxic centre fails, respiration becomes slow and then apneustic in type, a long inspiratory tonus is followed by a few short failing apneuses. Very soon gasps alone occur and death results. If however...the vertebrals are freed before gasping ceases, recovery takes place in the reverse order. Gasps give place to short and then to long apneuses; by periodical inhibition of these, slow respiration and soon normal breathing result." The arteries clamped by Lumsden were the carotids and vertebrals. My experience agrees with that of Hill in that the majority of cats survive the ligature of the arteries owing to the anastomosis which exists or becomes established through the other branches of the subclavians. In my experience too, respiratory effects if they are forthcoming at all always begin to manifest themselves within a second or two of the quadruple ligature. In those cases in which any delay occurred subsequent investigation showed that the occlusion had not been complete. Respiration always ceases and recovers in the ways which I have described. I have never seen either after occlusion or during recovery

a form of breathing which Lumsden calls apneusis. How the chest comes to stop in full expiration has been already described and illustrated in Fig. 10 where the actual movement was recorded. It is true that the return of respiration on re-opening an artery may take the form of sudden gasping inspirations. This fact might at first sight appear to be evidence in favour of a separate gasping centre such as Lumsden postulates. But these gasps instead of giving way to another form of respiration soften down regularly into ordinary quiet breathing. In some instances a more complex phenomenon is seen. Respiration recovers first in the form of deep gasps. On re-opening an artery the gasps quicken and between the gasps appear a normal respiration so that gasping and breathing take place alternately. Then there occur two breaths to a gasp, then three breaths and so on. Meanwhile the gasps diminish in depth progressively and themselves become gradually converted into normal respiration taking their place in the regular sequence. These results, I think, are evidence that gasping is to be regarded as a modification of the normal respiratory beat and not a form of respiration due to the uncontrolled action of a separate centre normally in abeyance. My experiments in fact lend no support to Lumsden's conception of the respiratory mechanism.

The respiratory centre is more readily damaged by anæmia than the vaso-motor. When the respiration has remained in abeyance during prolonged occlusion its resumption when blood is restored is often delayed though the blood-pressure recovers immediately. When the circulation and respiration are so depressed that artificial respiration has to be resorted to blood-pressure is always the first to recover.

Differences in different species. A word is necessary about the differences shown in the rabbit, cat and dog. In the cat it is, in my experience, despite Hürthle's evidence to the contrary, a matter of considerable difficulty to obtain a satisfactory record of the Circle pressure, owing to the extreme fineness of the internal carotid in this animal. I have attempted to take a record from the upper end of the vertebral but the results have not been satisfactory owing, I believe, to the many branches given off by this artery before it terminates in the basilar. I have not succeeded in the cat in controlling the rate of respiration by controlling the medullary blood-supply. As already stated it is more difficult to disturb function in the cat than in the rabbit owing to the freer collateral circulation which the cat possesses. In the dog it is impossible to do so. When in this animal the carotids and subclavians together with all their branches are clamped the Circle pressure is still remarkably high owing

to the very free anastomosis by way of the intercostal and spinal arteries. I see no reason for believing that there is any real difference in the central mechanism of respiration in these three species. Such differences in effects produced are due I believe to differences in blood-supply.

III. *The effect of lactic acid upon the anæmic medulla.*

It has often been stated that under special conditions the respiratory centre can be stimulated by lactic acid arising within it through deficient oxygen supply. Starling(9), for instance, quotes an experiment in which a rabbit was exposed to a low oxygen tension. On subsequently giving oxygen the disappearance of the hyperpnœa was immediate. Since exposure to low oxygen tension for a short time does not cause any lactic acid to appear in the blood the conclusion is drawn that if lactic acid were the cause of the hyperpnœa it must have been produced in the centre. Again, the fact that the rise in alveolar CO_2 after breathing an atmosphere deficient in oxygen was initially rapid suggested to Haldane and Poulton(10) that "most of the lactic acid which excited the centre was formed in the centre itself," and that the acid was either rapidly oxidised or washed away by the more alkaline blood which circulates very rapidly through the centre. Then there is the well-known hypothesis advanced by Douglas and Haldane(11) to explain the Cheyne-Stokes respiration which sometimes follows the apnœa due to excessive breathing. The centre suffering from oxygen want owing to the apnœa is stimulated by the lactic acid formed within it. "It is probable," write Haldane and Poulton, "that when the amount of oxygen is extremely low lactic acid is produced in the centre itself. It is perhaps the formation of lactic acid in the respiratory centre which actually terminates the apnœa after forced breathing." All these statements rest upon two assumptions; first, that lactic acid appears in the centre under the circumstances of the experiments; second, that were lactic acid so produced an anoxæmic centre would be capable of being stimulated by it. Neither of these assumptions has ever been proved. The first rests, apart from the post-mortem formation of acid in the brain, only upon the very dubious experiments of Langendorff(12). That the second assumption is not warranted is shown in Fig. 14. The record is taken from the rabbit prepared in the usual way. As the result of closure of the fourth artery (at A) the blood-pressure rose and reached a level of about 110 mm. while respiration stopped, in this case without any preliminary hyperpnœa, except for a few occasional gasps. At B, 7 mgms. lactic acid in 0.7 Ringer (previously boiled to exclude oxygen in solution) was injected

brainwards into one of the carotid arteries. There is no doubt that the lactic acid reached the medulla for there occurred the momentary inhibition of the heart which is liable to occur whenever anything is injected into the carotid. This was followed immediately by a further rapid rise of blood-pressure to 140 mm. The obvious super-imposition of this rise upon the first, following as it does directly upon the injection, must point to stimulation of the vaso-motor centre by the acid. Mathison (13) produced vaso-constriction by injection of lactic acid into the carotid, the other arteries being open. He therefore believed that the vaso-motor centre could be stimulated by lactic acid produced within it. My experiments support his contention. At any rate it is clear that an anæmic centre no less than one which has a free blood-supply can be stimulated by lactic acid.

In striking contrast with the vaso-motor is the respiratory centre. There is no reason to believe that injected substances diffuse with more difficulty into the respiratory centre than into the vaso-motor centre yet the former shows no sign of stimulation. It remains quiescent except for the gasps at regular intervals which had set in before the lactic acid was injected. At *C* the carotid was momentarily opened. Note the sudden fall in pressure and resumption of respiration to be stopped once more upon closure of the artery at *D*. The rapidity of respiration during this period may have been due, in part at least, to the presence of the injected acid in the medulla. At *E* blood was again admitted. This experiment therefore shows that the respiratory centre when quiescent from want of oxygen completely fails to respond to the presence of lactic acid in amount sufficient to stimulate the vaso-motor centre. It is therefore very unlikely that the centre would respond to lactic acid produced within it. This experiment shows incidentally one more point of difference between the respiratory and vaso-motor centres.

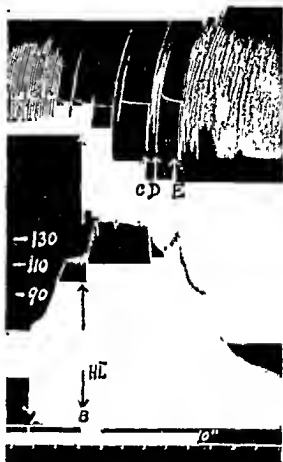


Fig. 14. Rabbit. Description in text.

Discussion of results.

The experiments described above have some bearing upon three questions affecting the respiratory centre. First, there is the effect of anoxæmia upon it. The balance of opinion upon this much-debated question is in favour of the view that want of oxygen is a stimulus to the centre. This view is based upon the results of experiments in which men and animals have been made to breathe atmospheres deficient in oxygen. Under these conditions, however, the centre is affected not only directly by the anoxæmia but also indirectly by the changed state of the blood due to anoxæmia in the whole body. In the experiments here described the rest of the body apart from the brain is not primarily affected and therefore any respiratory changes noted must be directly produced. The two kinds of experiment further differ in the fact that while in the breathing experiments there is brought about (though only primarily) a pure deficiency in oxygen, in these experiments what is altered is the quantity of blood. It is reasonable however to assume that reduction in quantity of blood produces its effects through insufficient oxygen. The very considerable reduction in blood to which the centre remains indifferent must involve a diminution in oxygen at least equal to if not more than that which obtains in the experiments in which a deficient oxygen supply is breathed. Unless the centre is indifferent towards a much diminished blood-supply at full oxygen saturation, while it is easily influenced by a normal blood-supply at low saturation, we are forced to the conclusion that anoxæmia has no direct effect upon the centre and that such effects as have been observed in the breathing experiments have been indirectly produced. The fact that in my experiments the respiratory centre responded vigorously to re-breathing of the expired air shows that the negative result could not have been due to the anæsthetic, for it is very unlikely that the centre would retain a high sensitiveness to CO_2 while its sensitiveness to oxygen-want was in any degree impaired.

The second point concerns the metabolism of the centre. No direct determination of the metabolism of the central nervous system has yet been satisfactorily performed. The indifference of the respiratory centre to diminution in its blood suggests that the group of cells of which this centre is composed has a comparatively low rate of metabolism.

Thirdly, as regards the nature of the respiratory centre. Those who have postulated that under conditions of defective oxygen supply there is accumulated in the centre an amount of lactic acid sufficient to

stimulate the centre, have argued from analogy with the appearance of lactic acid in muscle stimulated artificially under anaerobic conditions. In doing so they have tacitly assumed that the respiratory centre is a reflex organ, which has activity forced upon it as in the case of muscle in the experiments of Fletcher and Hopkins. But the fact that the main effect of insufficient blood upon the centre is diminished or suppressed function is evidence in favour of the view that the centre is essentially automatic though subject to modification by afferent impulses. The amount of lactic acid which at most could be produced within it would therefore be equivalent not to the amount formed in stimulated muscle but to the extremely small amount found in resting (anaerobic) muscle.

The most striking fact brought out in the above experiments is the contrast between the properties of the respiratory centre and those of the vaso-motor together with the cardio-inhibitory centre. The respiratory centre while very sensitive to the amount of CO_2 or the hydrogen ion concentration of the blood is insensitive to the quantity of blood, while the vaso-motor centre is far more sensitive to the quantity of blood than to the acidity; the respiratory centre is paralysed by extreme anæmia, while the vaso-motor centre is strongly stimulated; finally the respiratory centre when quiescent from extreme anæmia is insensitive to the injection of acid, a procedure which excites the vaso-motor centre to further activity. The two groups of cells which control the two great functions of respiration and circulation behave towards nutritional disturbances in a diametrically opposite manner. This fact is sufficient to show that phenomena relating to the incomplete metabolism of excised muscle are not necessarily the same in the nervous centres.

CONCLUSIONS.

1. Both the vaso-motor and cardio-inhibitory centres are directly stimulated by a slight diminution in their blood-supply. The resulting inhibition checks and sometimes annuls the rise of pressure which vascular constriction tends to produce.

2. Rarely the pressure in the Circle of Willis can be raised by the occlusion of a cerebral artery.

3. The respiratory centre is totally insensitive to diminution in its blood-supply until this diminution becomes extreme.

4. There is a certain critical Circle pressure varying between 18 and 6 mm. in different individuals, at which the respiratory centre is paralysed and the vaso-motor centre strongly stimulated. Stimulation of the cardio-inhibitory centre immediately follows.

5. The apnœa is sometimes preceded by transient hyperpnœa but this is not caused by defective oxygenation of the centre since it does not occur when the reduction of blood-supply is gradual.

6. When the reduction in blood-supply is gradual the blood-pressure rises but slightly owing to the fact that the cardio-inhibitory centre is stimulated simultaneously with the vaso-motor centre.

7. At low cerebral pressures the rate of respiration can in the rabbit be controlled by regulating the blood-supply.

8. Evidence is presented which suggests that under conditions of defective nutrition discharges from the respiratory centre may suffer a decrement and be suppressed somewhere on their way to the periphery.

9. Gasping is a modification in the nervous mechanism acting as a whole and is not due to the action of a separate centre.

10. When the medulla is completely or almost completely anæmic, injection of lactic acid directly into a cerebral artery stimulates the vaso-motor centre but is without effect upon the already paralysed respiratory centre.

11. Hæmorrhage is not attended with any increase in breathing until a very low pressure is reached. Hyperpnœa of variable degree then occurs.

12. The differences in the results found in rabbits, cats and dogs are due to differences in blood-supply.

13. The metabolic processes occurring in the respiratory centre play no part in controlling the rate and depth of respiration.

The expenses of this research were partly defrayed by a grant from the Government Grant Committee, Royal Society.

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OBSERVATIONS ON THE TAKING UP OF CARBON MONOXIDE BY THE HÆMOGLOBIN OF THE SPLEEN.

BY A. HANAK AND J. HARKAVY.

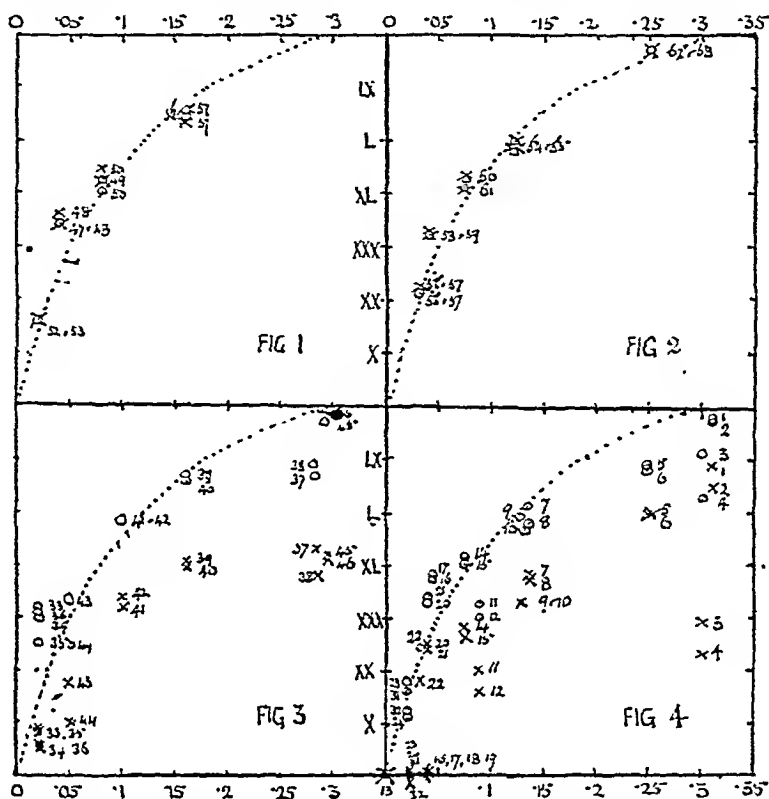
(From the Physiological Laboratory, Cambridge.)

THE present research is a continuation of that published by J. and H. Barcroft⁽¹⁾ who showed that on exposure of rats to an atmosphere which contained carbon monoxide the gas was rapidly taken up by the blood of the general circulation, but only slowly penetrated into the hæmoglobin of the spleen. They further showed that when in the spleen pulp the CO tended to remain there after it had disappeared from the general circulation. In their experiments the rats were in carbon monoxide at most for an hour and this time did not prove long enough for an equilibrium to be attained between the CO in the spleen pulp and that in the general circulation. Our first object was to ascertain the length of time taken for the attainment of this equilibrium. The method used was generally speaking the same as that described in the paper quoted and therefore need not be gone into, except where some special point arises. It became clear that our experiments would have to stretch over much longer time than those of our predecessors and therefore that much greater care would be necessary to maintain a constant concentration of CO in the chamber. The reason is as follows. Suppose the percentage of CO in the chamber were dropping, a false and premature equilibrium between the CO in the spleen and that in the blood would be established, not because the hæmoglobin of the spleen was gradually acquiring the gas, but because that of the blood was losing it.

Rigorous tests were therefore made of the capacity of the chamber to retain a given concentration of gas for a considerable time. The following is a sample—calculated concentration of CO put into chamber 0.165—hourly analyses made between the 11th and 18th hours after the concentration was set up gave results which varied between 0.160 and 0.166, the final one 0.163, thus the chamber retained all the CO so far as the experimental method of Haldane's apparatus could be expected to indicate.

Figs. 1 and 2 show the result of eighteen experiments in each of which

two rats were exposed to the concentration of carbon monoxide stated as the abscissæ for nine and six hours respectively, this series shows (a) that the hæmoglobin in the spleen is as fully saturated as that in the blood



Figs. 1-4. Abscissa=%CO in air, Ordinate=%CO hæmoglobin in \odot general circulation \times spleen pulp. Guinea pigs, times of exposure in chamber Fig. 1, 9 hours; Fig. 2, 6 hours; Fig. 3, 4 hours; Fig. 4, 2 hours.

after six hours' exposure and (b) that no difference can be determined between the saturation either of the blood or of the spleen pulp by extending the experiment to nine hours.

If on the other hand the time be shortened to four hours (Fig. 3) a chart is obtained of a different character. The saturation of the hæmoglobin in the spleen is in every case less than that in blood by quite a considerable amount. Speaking generally the deficit is about the same at all parts of the curve: this is only a very rough generalisation but it is sufficiently accurate to point the contrast with a probable condition namely that the deficit would be proportional to the absolute saturation. Any scheme which one might visualise, of dilution of the spleen pulp

with the circulating blood, and actuated by regular rhythmic contractions of the spleen, however slow and shallow, would tend towards the deficit being proportional to the concentration.

The comparison of Figs. 2 and 3 shows that the time taken for the spleen pulp to attain equilibrium with the blood is between four and six hours in resting guinea pigs.

By far the most exhaustive set of experiments was the series carried out on guinea pigs at two hours' exposure. Here two new features appear: (a) the blood saturation at the higher points falls below the line obtained in the other experiments, this point will be referred to later, and (b) the spleen saturations at CO pressures of below 0.05 p.c. of an atmosphere are in seven cases (Exps. 16-19, 23, 24, 31) nil and in one case (32) a minus quantity presumably due to an error in reading the reversion spectroscope. There are other experiments in the same region in which positive saturations are obtained in the spleen (20, 21 and 22) but the following statement covers the facts here presented:—in no case in which the blood saturation is below 24 p.c. is there any evidence of carbon monoxide having reached the spleen pulp in two hours in guinea pigs.

Exp. 13 is a control in which the animal was not put in the chamber and it appears on the chart as an isolated experiment. In point of fact a control of this kind was carried out for every pair of animals, thus there are over thirty experiments in guinea pigs alone to prove that the reading obtained from the hæmoglobin of spleen pulp in the ungassed animal is the same as that obtained from the hæmoglobin blood and therefore that the apparent absence of CO in the spleens of the animals noted, was not due to some systematic error which would produce a similar discrepancy in an animal whose blood contained no CO.

These facts seem to show that in guinea pigs at rest the spleen is practically shut off from the general circulation, and that the rhythmic contractions which are so constant a feature of plethysmographic tracings of the dogs' and cats' spleens are practically non-existent in the normal living guinea pig, when at rest, and possibly are due to experimental irritation of the spleens even in the animals on whose organs they have been observed.

A series of experiments was performed on rats very similar to the two hour series on guinea pigs and with much the same result, on the whole the figures both for spleen and blood tended to show a higher proportion of carboxyhæmoglobin at any given pressure of carbon monoxide but the deficit between the spleen and the blood was, as in the guinea pigs, usually about 15-20 p.c. There were two experiments in

which the CO hæmoglobin in the blood amounted to 28 and 24 p.c. respectively and in which no CO was found in the spleen. Twenty-four rats were used in this series.

In a few cases rats were kept some time after removal from the chamber and their bloods and spleens compared with those of animals killed in the chamber: the readings in this as in other experiments were made in duplicate and it will serve to show the sort of concordance which was obtained.

Rats 1, 2 and 3 were placed in the chamber for two hours, the concentration of CO was 0.145 p.c. 1 and 2 were then killed and 3 allowed to survive for another hour in air free from CO, the following are the saturations with CO:

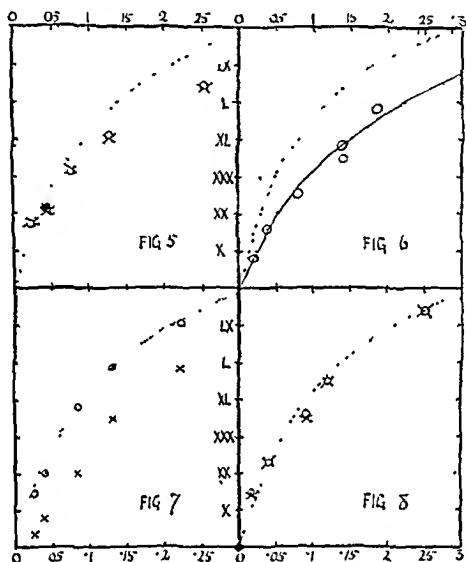
Rat	Observer	Blood	Spleen
		%	%
1	Hanak	50	34
	Harkavy	50	36
2	Hanak	53	32
	Harkavy	51	38
3	Hanak	16	16
	Harkavy	17	18

Of greater interest than the experiments done on rats are those done on rabbits. For their initiation we have to thank Dale and Burn who made a suggestion for testing our view, that the spleen pulp was largely out of the circulation unless the spleen were made to contract by some special stimulus. It was the injection from time to time of a dose of adrenalin into the ear vein, in which case contractions would follow and so in each relaxation mix the spleen pulp up with corpuscles from without. The series was never pushed to completion because the first few experiments gave us the key to what seemed a more satisfactory control, revealing the fact that in half-hour exposures, although if the animal is kept quiescent the deficit in CO between the hæmoglobin of the spleen the general circulation is considerable, yet the spleen and the systemic circulation rapidly get into equilibrium if the rabbit is handled in such a way as to make it "kick about" as rabbits do.

In Figs. 7 and 8, which illustrate the point, the exposures were half-hour ones; in Fig. 7 the rabbits were allowed to stay very quiet, in Fig. 8 they were handled, the bloods contained the same amount of CO in each case, relatively to the dose, but whereas in Fig. 7 the spleens show a deficit of CO hæmoglobin of 15 p.c. (more or less) in Fig. 8 the spleens and the bloods are equally saturated. A single comparison of 15 minutes' exposure showed the difference between the animal which

was handled and that which was not, the figures are as follows. The concentration of CO was .083 p.c.

Rabbit 1 handled, arterial blood 27 % sat. spleen 21 %
 " 2 quiet, " " 16 % " " 0 %



Figs. 5-8. Ordinate and Abscissa as in Figs 1-4 Exposures. Fig 5, rabbits quiescent, 1 hour, Figs 7 and 8, $\frac{1}{2}$ hour rabbits; 7, quiescent, 8, active; Fig 6, guinea pigs, equilibrium between blow and atmosphere, dotted line in animals, thick line in vitro.

This experiment shows some points of contrast with the half-hour experiments. The arterial blood has not in 15 mins. had time to attain equilibrium with the air which would mean about 35 p.c. saturation, but the blood of the rabbit which exercised itself violently as the result of being handled contained considerably more CO than that of the quiet rabbit. The second point is that here again we have a spleen which like those of some guinea pigs contained no carbon monoxide. Thirdly, whilst there is a great difference between the CO saturations of the hæmoglobin in the two spleens, even that of rabbit 1 had not quite as much CO as the blood.

This comparison is set out approximately for guinea pigs in Fig. 6—approximately, because the guinea pigs used for the equilibration experiments were not the same animals as those used for the absorption of CO in vitro, and there are slight differences for different animals as has been shown for rats, men, etc., by Douglas, Haldane and Haldane(3). Each point on the equilibration curve represents a determination on the blood of a different animal. The pressure of oxygen in the alveolar air is of course calculable by the method suggested by Haldane and Lorrain Smith(4) and indicate an alveolar oxygen pressure of 11–12 p.e. of an atmosphere in guinea pigs. A comparison between Fig. 6 and Figs. 1–4 will show that in certain cases the blood-points fall below the dotted line, indicating that the alveolar pressure approximates more nearly to the atmospheric. These points are usually with high pressures of CO in the atmosphere and might, no doubt, be interpreted as the result of oxygen secretion, but they could in most instances be explained on the assumption that the animals were rendered dyspnoic, which in fact they often were. We made no actual tests of the degree of dyspnoea.

SUMMARY.

1. The observation is confirmed that when rats are placed in atmospheres containing carbon monoxide, the hæmoglobin in the blood comes into equilibrium with that gas much more rapidly than it does with the hæmoglobin of the spleen pulp. This observation is extended to guinea pigs and rabbits.

In the case of guinea pigs, between four and six hours may elapse before the hæmoglobin in the spleen pulp attains equilibrium with that in the blood, in the case of rabbits over two hours may elapse—a fact which suggests the almost complete occlusion of the blood stream from the spleen, and the reduction of the rhythmic contractions to something less than normal.

When rabbits are lifted and “kick about” the CO goes into the spleen very rapidly so that the spleen pulp may become charged with the air which was in the atmosphere as great a percentage of CO hæmoglobin as it might be with the air which was in the atmosphere after two hours.

It is also shown that under such circumstances stimuli passing either directly to the spleen, or to the adrenals, produce a considerable increase in the contraction of the spleen, whether these are rhythmic or not. The second point is that the spleen of the guinea pig contracts considerably more than that of the rat or rabbit. A great difference between the spleen of the guinea pig and that of the rat or rabbit is exposed to air containing

CO, for long enough to establish equilibrium, the general law is observed,

$$\frac{[\text{COHb}]}{[\text{O}_2\text{Hb}]} = K \frac{[\text{CO}]}{[\text{O}_2]},$$

as between the arterial blood and the alveolar air, there are cases with the higher concentrations of CO in which the saturation of the arterial blood is less than the law indicates. The calculated alveolar pressure of oxygen is as a rule about 12-13 p.c. of an atmosphere.

We are much indebted to Mr Barcroft for suggesting this research and for help in carrying it out. We should like to take this opportunity of thanking Professor Langley for giving us the facilities of his laboratory, and the Medical Research Council to whom much of the apparatus used in this research belonged.

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NOTE BY J. BARCROFT.

Professor Langley in criticising the above kindly pointed out to me that the spleens of different animals differ greatly in the amount of muscular tissue contained in the capsule and that the particular animals used were all such as had rather little muscle. As Drs Harkavy and Hanak had left Cambridge I performed with the help of Miss Sands an experiment on cats, which have very muscular spleens. The scheme of the experiment was as follows. A concentration of 0.14 p.c. CO was "put up" in the chamber in which were five kittens, and also myself. I was breathing air piped to "respiratory" valves from outside so as not to imbibe CO. A kitten was killed by decapitation so sudden as to be instantaneous, at each of the times stated below and the CO in the bloods and spleens pulp analysed:

Time from zero (minutes)	5	10	20	40	60
% COHb { Blood	20	39	56	61	63
% COHb { Spleen pulp	—	0	29	52	58

affording a general confirmation on kittens of the experiments on rats, guinea pigs and rabbits.

RENAL FUNCTION IN SUMMER FROGS AND WINTER FROGS¹. BY J. DE HAAN AND A. BAKKER.

(From the Physiological Laboratory of the University of Groningen.)

It is a well-known fact, that frogs pass in their urine a large quantity of water which is taken up with the skin, and in this way maintain their water equilibrium. According to Clark(1), the tubules of the frog's kidney do not absorb water, but since the urea in frog's urine is more concentrated than in the blood, there must, on Cushny's theory, be some water absorption. As, furthermore, this urine contains only traces of the blood salts, the fluid absorbed must be a hypertonic salt solution, as was suggested by Parnas and Prilecki(2).

It has been observed, that together with this abundant elimination of water, there is found a somewhat difficult and retarded excretion of dyes in the frog. Von Möllendorff(3) speaks of a decreased permeability for dissolved dyes in the frog's kidney, as compared with that of the kidney in various mammals: thus diffusible dyes should remain much longer in the frog's body (several days) than in mammals (some hours).

In the present research we offer the results of some experiments which were undertaken to see how far the frog's kidney really differs from the mammalian. We compared the elimination of dyes in the frog, with the excretion in the same animal of other substances such as urea and of water. In earlier work on rabbits(4) it was concluded from comparative estimations of dye-stuffs in blood and urine, that absorption of water in these animals must take place in a much higher degree than would appear from the concentration ratio of urea; and that Cushny's theory of glomerular filtration is not tenable, unless one allows that a considerable part of the plasma colloids pass into the glomerular filtrate.

It was interesting to determine the behaviour of the frog's kidney under the same conditions, since in this animal the urine, as compared with the blood, is but little concentrated.

Methods. Frogs (*Rana esculenta*), living in a glass vessel in water, were treated with dyes (fluorescein potassium, trypan blue), by injection into the dorsal lymph sac; urine was taken by putting a small glass tube into

¹ A brief communication of these researches was delivered at the International Physiological Congress at Edinburgh, 1923.

TABLE III. Concentrations of urea and fluorescein kadium, simultaneously determined in urine, blood, and aqueous humour, some hours to one day after subcutaneous injections of 1.5 c.c. fluorescein kadium.

Month	Temp. of environment	Refraction of blood-plasma	Concentration dye blood-plasma	Proportion concentr. dye blood-plasma: conc. dye urine	Concentration dye urine	Proportion concentr. dye aqueous humour: conc. dye urine	Concentration dye aqueous humour	Concentr. urea in blood-plasma (mgr. in 100 c.c.)	Proportion concentr. urea blood-plasma: conc. urea urine	Concentr. urea in urine (mgr. in 130 c.c.)
July	25° C.	—	·00045 = 1 : 2200	1 : 1.4	·00062 = 1 : 1600	—	—	25	1 : 1.8	44
"	"	—	·00125 = 1 : 800	1 : 1.6	·002 = 1 : 500	1 : 25	·00008 = 1 : 12500	34	1 : 1.6	44
"	"	1.3390	·001 = 1 : 1000	1 : 1	·001 = 1 : 1000	1 : 25	·00004 = 1 : 25000	34	1 : 1.2	40
"	"	1.3435	·00125 = 1 : 800	1 : 0.72	·0009 = 1 : 1100	1 : 14.5	·000062 = 1 : 16000	19	1 : 3.5	68
"	"	1.3372	·00105 = 1 : 950	1 : 0.95	·001 = 1 : 1000	1 : 5	·0002 = 1 : 5000	42	1 : 1.2	50
Sept.	26° C.	1.3475	·00016 = 1 : 6250	1 : 2.9	·00045 = 1 : 2200	1 : 30	·000014 = 1 : 70000	12.5	1 : 3.2	40
"	10° C.	1.3420	·0025 = 1 : 400	1 : 0.32	·0008 = 1 : 1250	1 : 8	·0001 = 1 : 10000	7.2	1 : 3	22
"	0° C.	1.3408	·0016 = 1 : 625	1 : 0.75	·0012 = 1 : 850	1 : 7.5	·00016 = 1 : 6250	10.8	1 : 3	32
Feb.	10° C.	1.3389	·0006 = 1 : 1660	1 : 0.66	·0004 = 1 : 2500	1 : 3.5	·00011 = 1 : 9000	40	1 : 2.5	100
"	10° C.	1.3428	·0009 = 1 : 1100	1 : 0.35	·00033 = 1 : 3000	1 : 4	·00008 = 1 : 12500	40	1 : 3.75	160
April	14° C.	—	·00059 = 1 : 1900	1 : 0.5	·00025 = 1 : 4000	1 : 6	·00004 = 1 : 25000	14.1	1 : 2	27.5

This table contains also the refraction values of the serum, indicating the protein content of the plasma. It is obvious that this protein content must be an important factor in regulating the quantitative relations of the free and the combined fraction of the dye.

DISCUSSION

We consider it to be definitely proved—and most recently by direct inspection in the experiments of Bieter and Hirschfelder(6)—that the dyes leave the kidney wholly by glomerular filtration, and from this standpoint we discuss the question of the passage of proteins through the glomerular membrane.

In a recent article by Marshall and Vickers(7) the opposite opinion is maintained. These authors claim that dyes are secreted by the tubules, an hypothesis also supported by Mayrs(8). They reject our contention that protein passes through the glomerular membrane.

We cannot enter into the details of this question here, our standpoint having been amply treated by us in a previous article (9), but we would point out that the concentration of phenol red, proved by M and V's experiments, is not inconsistent with the passage of dye and protein through the glomerular membrane. When the passage through the glomerulus is sufficiently slow, the whole of the water might be absorbed and, naturally, concentration of the dye would occur in the lumina of the tubules, and it might partly be taken up by the cells. The fact that in the case of more colloidal dyes as trypan blue any accumulation of dye in the tubule cells fails, seems to us a direct argument against the hypothesis of a picking up of the dye by the tubule cells from the blood.

Trypan blue, as in mammals, is totally adsorbed to the plasma colloids. An ultra-filtrate of frog's plasma, with *e.g.* 1/1500 trypan blue, does not contain even a trace of the dye. In the aqueous humour the dye totally fails or only negligible traces are present. Yet the urine, even in winter frogs at a low temperature, contains the dye. It seems impossible to account for this fact, unless we accept the view that the glomerular membrane is not altogether impermeable for colloids.

There is one circumstance which at first sight offers the possibility of another explanation. From Table III one sees that the protein content of frog's plasma varies between wide limits. Low protein values obviously will facilitate the water excretion, and one might suppose, that in summer frogs with their enormous water production, the protein content as a rule might in compensation be very low. Table III shows, however, that there is no definite rule. Now it is remarkable, that after injection of trypan blue one finds a very low protein content at least during the first day. Six frogs after subcutaneous injection of trypan blue showed next day in their blood plasma refraction values from 1.3365–1.3375, in the case of an intraperitoneal injection four hours after the injection we found values

TABLE III. Concentrations of urea and fluorescein kalium, simultaneously determined in urino, blood, and aqueous humour, some hours to one day after subcutaneous injections of 1.5 c.c. fluorescein kalium.

Month	Temp. of environment	Refraction of blood-plasma	Concentration dye blood-plasma	Proportion conc. dye blood-plasma: conc. dye urine	Concentration dye urine	Proportion conc. dye aqueous humour: conc. dye urine	Concentration aqueous humour	Concentr. urea in blood-plasma (mgr. in 100 c.c.)	Proportion conc. urea blood-plasma: conc. urea urine	Concentr. urea in urine (mgr. in 130 c.c.)
July	25° C.	—	0.0045 = 1 : 2200	1 : 1.4	0.0062 = 1 : 1600	—	—	25	1 : 1.8	44
"	"	—	0.0125 = 1 : 800	1 : 1.6	0.002 = 1 : 500	1 : 25	0.0008 = 1 : 12500	34	1 : 1.6	44
"	"	1.3390	0.0125 = 1 : 800	1 : 1	0.001 = 1 : 1000	1 : 25	0.0004 = 1 : 25000	34	1 : 1.2	40
"	"	1.3435	0.0125 = 1 : 800	1 : 0.72	0.0009 = 1 : 1100	1 : 14.5	0.00062 = 1 : 16000	19	1 : 3.5	68
"	"	1.3372	0.0125 = 1 : 800	1 : 0.95	0.001 = 1 : 1000	1 : 5	0.0002 = 1 : 5000	42	1 : 1.2	50
"	"	1.3475	0.0105 = 1 : 950	1 : 2.9	0.0045 = 1 : 2200	1 : 30	0.00014 = 1 : 70000	12.5	1 : 3.2	40
Sept.	26° C.	1.3420	0.0016 = 1 : 6250	1 : 0.32	0.0008 = 1 : 1250	1 : 8	0.0001 = 1 : 10000	7.2	1 : 3	22
"	10° C.	1.3408	0.0025 = 1 : 400	1 : 0.32	0.0008 = 1 : 1250	1 : 8	0.0001 = 1 : 10000	7.2	1 : 3	32
"	0° C.	—	0.0016 = 1 : 625	1 : 0.75	0.0012 = 1 : 850	1 : 7.5	0.00016 = 1 : 6250	10.8	—	—
Feb.	10° C.	1.3389	0.0006 = 1 : 1660	1 : 0.65	0.0004 = 1 : 2500	1 : 3.5	0.00011 = 1 : 9000	40	1 : 2.5	100
"	10° C.	1.3428	0.0009 = 1 : 1100	1 : 0.35	0.00033 = 1 : 3000	1 : 4	0.00008 = 1 : 12500	40	1 : 3.75	150
April	14° C.	—	0.0052 = 1 : 1900	1 : 0.5	0.00025 = 1 : 4000	1 : 6	0.00004 = 1 : 25000	14.1	1 : 2	27.5

This table contains also the refraction values of the serum, indicating the protein content of the plasma. It is obvious that this protein content must be an important factor in regulating the quantitative relations of the free and the combined fraction of the dye.

DISCUSSION

We consider it to be definitely proved—and most recently by direct inspection in the experiments of Bieter and Hirschfelder(6)—that the dyes leave the kidney wholly by glomerular filtration, and from this standpoint we discuss the question of the passage of proteins through the glomerular membrane.

In a recent article by Marshall and Vickers(7) the opposite opinion is maintained. These authors claim that dyes are secreted by the tubules, an hypothesis also supported by Mayra(8). They reject our contention that protein passes through the glomerular membrane.

We cannot enter into the details of this question here, our standpoint having been amply treated by us in a previous article (9), but we would point out that the concentration of phenol red, proved by Marshall and Vickers' experiments, is not inconsistent with the passage of dye and protein through the glomerular membrane. When the passage through the glomerulus is sufficiently slow, the whole of the water might be absorbed and, naturally, concentration of the dye would occur in the lumina of the tubules, and it might partly be taken up by the cells. The fact that in the case of more colloidal dyes as trypan blue any accumulation of dye in the tubule cells fails, seems to us a direct argument against the hypothesis of a picking up of the dye by the tubule cells from the blood.

Trypan blue, as in mammals, is totally adsorbed to the plasma colloids. An ultra-filtrate of frog's plasma, with e.g. 1/1500 trypan blue, does not contain even a trace of the dye. In the aqueous humour the dye totally fails or only negligible traces are present. Yet the urine, even in winter frogs at a low temperature, contains the dye. It seems impossible to account for this fact, unless we accept the view that the glomerular membrane is not altogether impermeable for colloids.

There is one circumstance which at first sight offers the possibility of another explanation. From Table III one sees that the protein content of frog's plasma varies between wide limits. Low protein values obviously will facilitate the water excretion, and one might suppose, that in summer frogs with their enormous water production, the protein content as a rule might in compensation be very low. Table III shows, however, that there is no definite rule. Now it is remarkable, that after injection of trypan blue one finds a very low protein content at least during the first day. Six frogs after subcutaneous injection of trypan blue showed next day in their blood plasma refraction values from 1.3365–1.3375, in the case of an intraperitoneal injection four hours after the injection we found values

such as 1.3350. At the same time the coagulation-time of the blood was considerably retarded.

We hope to investigate more closely this phenomenon, which seems to us of considerable importance. It seems probable that it indicates a way in which the frog automatically secures its water-elimination in conditions in which this might seriously be menaced (as after trypan blue injection).

In this place we will only treat the phenomenon as it affects the question of the passage of proteins through the glomerulus. As to this, one might be inclined to think that the lowering of the protein content to about 1 p.c. might set free part of the trypan blue. Ultra-filtrating, however, such a serum with trypan blue shows that even then not a trace of the dye passes, so that a passage of protein dye compound is required in this as in other cases.

There is another remarkable phenomenon after injection of trypan blue: in winter frogs after one day, in summer frogs after several days, the excretion of the dye stops totally, though in the blood the dye is still present in large quantities, at least in winter frogs. This will partly be caused by the fact that the more diffusible parts of the dye are eliminated first, and in part by a decreasing permeability of the dye as a consequence of progressive aggregation of dye molecules. After intraperitoneal injection the diffusion of the dye into the whole body goes on very quickly; the excretion of the dye soon reaches very high values; after subcutaneous injection, the dye disperses much more slowly, and then a much lower concentration of the dye in the urine is found even when the dye concentration in the blood is at its maximum; this indicates that the diffusing properties of the dye gradually decrease during its stay in the body.

In the case of trypan blue the concentrations of the dye in blood and urine cannot usually be compared, as the dye in the urine is found in a reddish modification. Table III, however, gives these values for fluorescein kalium. This brings out a striking difference between frogs and mammals. In the mammal (rabbits) the ratio $\frac{\text{dye urine}}{\text{dye blood}}$ is considerably higher than the ratio $\frac{\text{urea urine}}{\text{urea blood}}$. In the frog we see the reverse (Table III). Still there is no reason to suppose that in the frog the dye is largely absorbed. Thus there is probably a concentration of the dye as well as of the urea in the formation of the urine, and we may conclude, therefore, that the concentration of the dye in the glomerular filtrate is considerably lower than in the blood; this reduced dye content in the glomerular filtrate will automatically arise if the filtrate is free of proteins. On the supposition that this is the case, we must compare the concentration of the dye in the urine with that in an ultra-filtrate of blood; as ultra-filtrating frog's blood is not very easy, we have taken the aqueous humour, which may be expected to give reliable values in this respect, if one takes care to allow sufficient time for diffusion. Then we see from Table III:

(1) In winter frogs and at a low outer temperature the concentration of the dye in the urine is markedly lower than in the blood; the ratios for $\frac{\text{urea urine}}{\text{urea blood}}$ (2-3.75) do not differ very much from the concentration derived from the ratio $\frac{\text{dye urine}}{\text{dye aqueous humour}}$ (3-8).

(2) In summer frogs, however, the ratio $\frac{\text{urea urine}}{\text{urea blood}}$ remains the same (1.2-3.5), the concentration of the dye in the urine however rises, so that the values obtained are very often larger than in the total blood, and the concentration as compared with the aqueous humour rises to a much higher amount (5-30). At the same time the concentration of urea in urine compared with the blood is nearly the same as in winter frogs. This is an indication, that the mass of absorbed water, the concentration, is not greater in summer frogs. The higher level of dye excretion, therefore, must be caused by something else; and it is obvious to make a greater permeability of the dyes responsible for it, in the sense that not only the free part of the dye, but also a part of the combined dye, in so far it is linked to smaller colloid molecules, is let through. Nevertheless, the permeability for proteins is far from reaching the degree, which must exist in rabbits, for the ratio $\frac{\text{dye urine}}{\text{dye blood}}$ remains always smaller than the ratio $\frac{\text{urea urine}}{\text{urea blood}}$, while in mammals the reverse occurs.

One might suppose that the difference between summer frogs and winter frogs had no connection with the question of passage of colloids, and that it was only caused by the fact that in winter frogs the free part of the diffusible dye only partly is let through. This supposition is not probable, as the dye is very diffusible, and is quite impossible for the case of trypan blue.

Still there remains the possibility that the combined dye, passing the glomerulus, is linked to something else than a protein. This possibility cannot be discarded; but we must remember, that by far the largest part, if not all, of the dye is combined to protein. Moreover, the way in which the dye accumulates in the tubules of the frog is the same as that found in mammals: within the lumen of the tubules of a frog one finds the same protein-like precipitate as in mammals.

Supposing for a moment that only a protein free glomerular filtrate could pass, and taking the aqueous humour for an ultra-filtrate, it follows that in summer frogs the urine would be concentrated about 20-30 times. On warm days a summer frog would then produce a 24 hours' quantity of $30 \times 50 = 1500$ c.c. glomerular filtrate, and from this 90 p.c. of the urea must be absorbed. How far this agrees with the circulatory relations

in the frog's kidney cannot be said with certainty. Bieter and Hirschfelder(6) come to a rough estimate of a concentration of about 12-16 times; as derived from the urea concentration it would at its most rise to about four times; probably it will be higher, but never reach values of 30. Our researches, therefore, lead to the conclusion, that in frogs the glomerular filtrate must approach much more to a real ultra-filtrate than in mammals, at least in winter time and at a low temperature. There must be present, however, traces of proteins even in winter frogs, and in summer frogs this amount of protein must be not inconsiderable. As long as the phenomena described above cannot be explained in another way, for which we see no possibility, one cannot escape from these conclusions.

The absence of albumen in the glomerular puncture fluid in Richards' method(10) is, it seems to us, in no real conflict with our view. All things point to the capillary permeability for protein being something largely varying. If one finds no protein in special experimental conditions, it does not mean, that in the normal animal under optimal circulatory conditions this substance is totally retained in the same way.

It would be premature to speculate on the cause of the difference in permeability between summer frogs and winter frogs. Possibly the whole is due to a markedly increased heart action and blood stream in summer frogs. We have seen that a difference in protein content of the plasma cannot be responsible for it.

SUMMARY.

(1) There is a marked difference as regards the elimination of water in summer frogs and winter frogs. Most of this is caused by a difference in outer temperature, a smaller part by some internal factor.

(2) Corresponding with the quantity of water excreted, the rate with which diffusible dyes are excreted is much higher in summer frogs than in winter frogs. Still the velocity of dye excretion in the frog, even in optimal conditions, remains behind the values for mammals.

(3) There exists a marked difference between frogs and mammals as to the relative concentration of dye and urea in the urine; in mammals the dye is more concentrated than urea compared with the relative blood concentration; in the frog the concentration values are larger for urea. This holds especially for winter frogs. Here, as compared with the dye concentration in the blood, the urine contains the dye in a more diluted condition; in summer frogs the concentration of the dye in the urine often surpasses the same in the blood plasma. The rate of con-

centration for urea remains nearly the same in winter frogs and summer frogs.

(4) This fact is accounted for if there is a much smaller permeability for proteins in the frog than in mammals; in winter frogs the glomerular filtrate may contain no proteins, whereas in summer frogs a not inconsiderable quantity of smaller protein molecules must pass the glomerulus.

(5) The experiments with trypan blue prove, that even in winter frogs the glomerular membrane cannot be totally impermeable to proteins.

(6) The refraction values, *i.e.* the protein content of the plasma in normal frogs vary between wide limits (1.3360–1.3470), both in summer frogs and in winter frogs. After injection of trypan blue one sees as a rule a decrease of this protein content to refraction values of about 1.3350–1.3370.

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THE CHANGES IN ALVEOLAR CO_2 PRESSURE AFTER
VIOLENT EXERCISE. BY J. B. S. HALDANE
AND J. H. QUASTEL, 1851 *Senior Exhibitioner*.

(From the Biochemical Laboratory, Cambridge.)

DOUGLAS and HALDANE(1) showed that the alveolar CO_2 pressure remained low for about an hour after intense exertion, and Christian-sen, Douglas and Haldane(2) that the CO_2 capacity of the blood was also lowered. Ryffel(3) demonstrated the presence of unusually large amounts of lactate in the blood, and Barr and Himwich(4) that the lactic acid entering it accounted for the change in its CO_2 capacity. It is generally admitted that the breathing at rest is so regulated as to keep the cH of the arterial plasma approximately constant. According to the results of Campbell, Douglas, Haldane and Hobson(5), and Lilien-strand and Arborelius(6), the changed activity of the respiratory centres compensates for 96 p.c. of the change in cH which would otherwise occur, according to Parsons, Parsons and Barcroft(7). 92 p.c. Barr(8) found very inadequate compensation during exercise, but observed a return of the cH to normality within the limits of error of his method, within a period sometimes as short as eight minutes after the end of the exercise, and long before the alveolar CO_2 had returned to normal. It is therefore justifiable, when once the excess of CO_2 produced during the exercise has been got rid of, to take the depression of the alveolar CO_2 below normal as a measure of the amount of extra lactic acid in the blood.

We carried out 22 experiments in each of which the subject ran up and down stairs at top speed. He then rested for a few minutes, after which pairs of inspiratory and expiratory alveolar CO_2 samples were taken by the method of Haldane and Priestley(9) at intervals of about five minutes. Three experiments were done on J. H. Q. and 19 on J. B. S. H. In all 821 samples were analysed. The flight of stairs used was 10 metres in height. In all the experiments except one on J. H. Q. it was ascended and descended five times. The time taken varied between 3 min. 30 sec. and 3 min. 2 sec., improving with training.

The alveolar CO_2 fell rapidly, reaching a minimum value about 15 minutes after the end of the exercise, and rising to a fairly steady value after a variable time later. The curves showing the recovery fall into three

classes. In the ten "good" experiments¹, of which two examples (Figs. 1 and 2) are given, the points approximate very closely to a straight line.

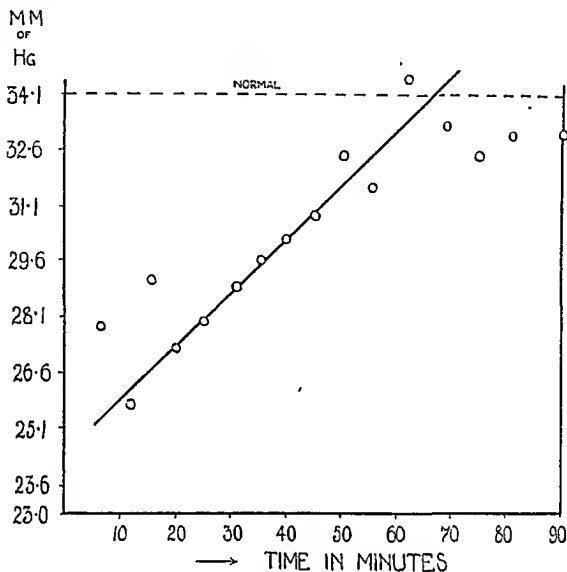


Fig. 1.

In the six "fair" experiments the approximation is less close, while the five "bad" experiments suggest no particular law. In no case was there the least approximation to any curve other than a straight line. The linearity tended to improve with practice. In order to test whether deviations from it were due to random causes we applied the following test. Lines were drawn through all the points which fell below the final value by more than one-quarter of the maximum depression, in such a way that half the observed points in any experiment lay on each side of the line. If there were any tendency, as might be expected, for the velocity of recovery to slow down progressively, the true curve would be concave downwards. In this case the points in the middle of the range would lie above the straight line. Actually 161 points lay in the ranges

¹ The experiments are classed as "good," "fair" or "bad," according to their approach to linearity.

considered. Of the $80\frac{1}{2}$ points in the middle sections $41\frac{3}{8}$ lay above the lines, $39\frac{1}{8}$ below. As we suspected that the lowest points might be for-

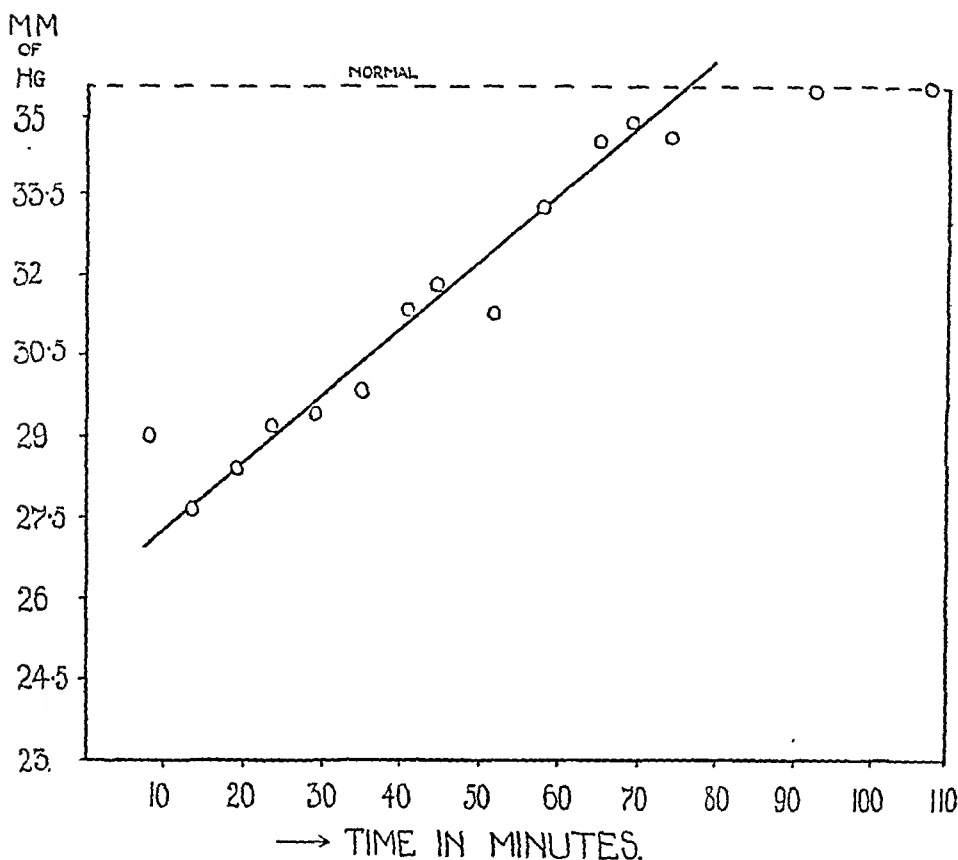


Fig. 2.

tuitously low, the same process was repeated, but extended only to the bottom point but one. One hundred and thirty-four points were considered. Of the 67 middle points, $31\frac{1}{4}$ lay above the line, $35\frac{3}{4}$ below, suggesting a slight concavity upwards. It is clear therefore that the deviations from linearity were at random, though there is doubtless curvature in the last quarter of the true recovery curve.

The results of the experiments are summarised in the table. The averages and probable errors are for the 19 experiments on J. B. S. H. The recovery rates were estimated by drawing a line as nearly as possible through the middle of the points on the recovery portion of each graph. The average in brackets for the rate of recovery is that of the "good" experiments only, which are more reliable. The mean maximum de-

Date 1922	Subject	No. of points	Linearity	Initial value mm. Hg.	Max. de- pression mm. Hg.	Recovery rate mm. Hg. per min.	Final value mm. Hg.
Oct. 21	Q.	13	B	40.0	6.9	.103	38.5
24	"	10	G	36.1	4.9	.180	36.2
26	"	8	F	35.4	4.8	.224	35.4
Nov. 2	H.	26	B	36.0	8.5	.074	34.0
4	"	17	G	35.7	8.1	.128	35.7
6	"	18	F	32.4	7.2	.140	31.2
8	"	22	G	32.1	10.3	.145	32.0
11	"	15	G	35.2	8.0	.218	35.5
13	"	20	F	35.3	10.0	.200	30.6
15	"	17	G	35.2	7.4	.130	36.6
1923							
Jan. 16	"	18	B	33.5	9.6	.180	31.9
Aug. 2	"	18	B	35.3	9.3	.185	35.6
4	"	21	G	34.5	6.9	.150	35.9
6	"	17	B	35.7	8.1	.165	35.9
8	"	20	F	35.8	10.7	.153	33.7
10	"	23	G	35.8	8.1	.101	36.1
13	"	24	F	32.6	9.1	.095	32.4
15	"	19	F	35.7	8.2	.138	34.4
17	"	22	G	34.4	8.8	.137	33.4
19	"	24	G	35.1	8.1	.114	33.5
21	"	21	G	35.0	7.5	.122	34.2
24	"	16	F	34.5	8.6	.161	33.7
Mean	H.	—	—	34.8 ± .8	8.6 ± .7	.144 ± .021 (.139 ± .020)	34.0 ± 1.2

pression of alveolar CO₂ was 8.6 mm. out of 34.8. As the present CO₂ dissociation curve of J. B. S. H.'s blood is not known it is impossible to calculate the exact amount of lactic acid which this represents, but assuming the usual ratio of 20 : 1 for combined and dissolved CO₂, and Bohr's(10) coefficient of solubility of .51, the combined CO₂ was reduced by 11.54-volumes p.c., or .00515 *M*, corresponding to an increase of .046 p.c. of lactic acid, or 2.8 grams in six litres of blood. This is a very small fraction of the whole amount left in the body, which according to Hill and Lupton(11) may exceed 100 grams. Clearly the muscles must contain membranes almost, though not quite, impermeable to lactic acid.

There is no clear indication of an effect of training either on the depression or the rate of recovery, the first of J. B. S. H.'s recovery rates being very unreliable. On August 15th and 21st a dose of 10 grams NaH₂PO₄·H₂O had been taken five hours before the run. This had no noticeable effect on either the speed of running, the lactic acid production, or the rate of recovery. This is, of course, no disproof of Embden, Grafé and Schmidt's(12) statement that it facilitates prolonged exercise by assisting the re-synthesis of lacticidogen, for the lactic acid is very likely converted first into carbohydrate.

The fact that, until a steady state is almost reached, the rate of

removal of lactic acid from the blood is constant, and therefore independent of its concentration, seems to prove two points. The removal rate does not depend on diffusion, nor on the amount of lactic acid available for some chemical reaction. Either of these would give a recovery curve concave downwards. It is clear that if the lactic acid (or rather lactate ion) diffuses out, the half-period of the diffusion process must be short, probably less than five minutes. The close analogy to the course of enzyme actions *in vitro* where there is an excess of substrate suggests that the limiting factor is the amount of some catalyst available. Barr and Himwich(4) showed that lactic acid was removed from the blood in passing through a resting arm, while the experiments of Embden and Kraus(13) show that the liver has the same power. But since, as Hill and Lupton(11) showed, intramuscular lactic acid can be removed after exercise at a rate of about 15 grams per minute, and the mean rate of removal from the blood of J. B. S. H. was only .045 gram per minute, it is obvious that at most only a minute fraction of the catalyst in the muscles is available for removing lactic acid from blood, and by no means certain that the muscles are directly concerned at all. This remains true even if there is a good deal of lactate in lymph and plasma in equilibrium with the blood. The muscle fibres can be no more permeable to lactic acid from without than from within.

SUMMARY.

The depression of the alveolar CO_2 after exercise can be used as a measure of the excess above normal of lactic acid in the blood. The amount of the latter after exercise and the rate of its removal have been determined. The rate is independent of the amount of lactic acid present, and is little affected by training or phosphate ingestion. The process of removal is vastly slower than that of intramuscular lactic acid.

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THE EFFECT OF CURARI AND DENERVATION UPON
THE ELECTRICAL EXCITABILITY OF STRIATED
MUSCLE. BY C. F. WATTS (*Research Student of Gonville
and Caius College, Cambridge*).

(*From the Physiological Laboratory, Cambridge.*)

THE relation between the strength and duration of the current needed to excite a tissue has been studied by Keith Lucas(1), Lapique(2), and others. The time constants of the strength-duration curve and in particular the "chronaxie" (i.e. the current duration at which the threshold strength of current is doubled) are generally considered as having fixed values for any one tissue. With nerve and heart muscle the results of various observers are in agreement, but in the case of skeletal muscle this is not so.

For the non-neural portion of a frog's sartorius Keith Lucas obtained a "chronaxie" of about .003 sec., while Lapique obtains a value of about .0002-.0005 sec. as the "chronaxie" of various skeletal muscles. The discrepancy between these results has been explained by the work of Jinnaka and Azuma(3) and H. Davis(4), who showed that the area of the stimulating electrode plays an important part in determining the "chronaxie." With a fluid electrode, they obtain a long value approximating to that found by Lucas with this type of electrode, but when an electrode of small stimulating area, such as the capillary pore electrode devised by Pratt, or the small silver chloride electrode used by Lapique, is employed, they find a short value of the same order as that obtained from nerve. This result makes it difficult, as Davis pointed out, to assign any definite "chronaxie" to the muscle, and must also be taken into account in any attempt at a physical explanation of the strength-duration curve.

The differing results of Lucas and Lapique are, however, of great interest in connection with the supposed change in the "chronaxie" of the muscle fibres observed under the action of curari, and after section and degeneration of the nerve fibres supplying them. In both cases the muscle becomes inexcitable through its nerve, and in both cases the "chronaxie" of the muscle, taken as a whole, is observed to lengthen.

How far is this change due merely to the fact that the stimuli formerly took effect on the intra-muscular nerve fibres, and now excite the muscle fibres themselves, and how far does it imply an actual change in the time constants of these muscle fibres? The nature of the change in the case of denervation is important in connection with the electrical testing of muscles, and the action of curari is a point of some theoretical interest.

Keith Lucas, using his fluid electrodes (5), found that the non-neural region of a frog's sartorius gave a simple curve with a "chronaxie" of $\cdot 003$ sec. He called this the α curve and regarded it as characteristic of the muscle fibre. In the neural region of the muscle he obtained complex curves, double or even triple in form. One of these complex curves had the same form as the α curve and of the other two, one, γ , had the same time constants as the curve obtained from the nerve trunk ("chronaxie" about $\cdot 0005$ sec.), and the other, β , had the shortest "chronaxie" of the three. Doses of curari just strong enough to abolish conduction from nerve to muscle caused the γ curve to disappear and slowed the time constants of the β curve. Slightly stronger doses abolished the β curve altogether, leaving the α curve unchanged. Lucas concluded from these results that the very rapid β curve represented the excitation of an intermediate substance between muscle and nerve—a conception agreeing in general terms with that which had been formed from the action of drugs.

According to this view curari does not affect the muscle fibre itself, but breaks the connection between nerve and muscle by its specific action on an intermediate substance localised in the region of the nerve endings and having a very short "chronaxie." Lapicque, using his small silver chloride electrodes, finds the "chronaxie" of normal nerve and muscle to have approximately the same value and he regards this "isochronism" of the two tissues as essential to conduction between them. The effect of curari is, from his point of view, to lengthen the "chronaxie" of the muscle fibre itself, and when this becomes about twice its normal value conduction fails owing to the "heterochronism" of nerve and muscle so produced. Failure of conduction is hence not due to any specific effect upon an intermediate substance but to an effect on the muscle fibre itself causing a slowing of its time constants. The nature of the effect of small doses of curari on the strength-duration curve of a muscle fibre is hence an important point in connection with the supposed mode of action of the drug and I have therefore investigated the strength-duration curves of normal, denervated, and curarised

muscles with special reference to the type of stimulating electrode employed.

Methods. (1) Fluid electrodes of the slot type were employed. Two slots of differing sizes were used, the area of one being 5×1 mms. and the other 3.5 times as great— 7.5×2.5 mms. The smaller slot just accommodates a normal sartorius. Both gave curves extending over a similar range for normal muscles but the threshold in the case of the larger slot was materially higher and more variable as the form of the current led in probably varied more than in the case of the smaller slot.

(2) For stimulating individual muscle fibres two Pratt electrodes were employed, one having a diameter of 3.5μ and the other being an ellipse $73 \times 44\mu$. (These pores were made by Dr H. Davis to whom I am indebted for their use.) The smaller pore was capable of stimulating one fibre only, but with the larger one this was not always the case as its dimensions are greater than those of an average muscle fibre ($20 \rightarrow 40\mu$). Hence the range of values obtained from it is relatively larger but in all cases the curves from muscles under similar conditions are of a similar type. Ag-AgCl electrodes were used for connection with the pore, and for the indifferent electrode which dipped in the Ringer in which the whole frog was immersed during this stage of the experiment. The pores were used on the muscle (sartorius throughout) *in situ* 1 hour after skin reflection and immersion of the frog in the bathing fluid. The sartorii were then dissected out and after leaving for $1-3\frac{1}{2}$ hours in the requisite bathing fluid the strength-duration curves were redetermined with the fluid slot electrode. The spring contact breaker and short-range pendulum designed by Lucas(1) were both used for obtaining currents of short duration. Currents ranging from $.042-0.00025$ sec. could thus be obtained.

Normal muscles. To describe a strength-duration curve (*e.g.* of the complex form shown in Fig. 1 D) it is not sufficient to note only the "chronaxie," and I have taken four points on each curve in comparing those obtained under different conditions. These are: (a) Threshold (θ), *i.e.* the least value of current of infinite duration which will excite; (b) the time of first rise of threshold, *i.e.* time at which the current required to excite is first greater than (θ); (c) the "chronaxie"; (d) the minimum time which a current of infinite strength must last in order to excite. This is called the Minimal Excitation Time. The form of the curves will not be affected by variation in (a), but will be altered by changes in (b), (c) and, to a less extent, (d). The following table gives the values obtained from normal muscles by the different techniques:

TABLE I. Normal muscles.

<i>Electrode</i>		Threshold (θ)	Time of 1st	Chronaxie	Min. excitation time
		Volts (a)	rise from (θ) Secs. (b)		
Small pore 3.5 μ dia.	(1)	.54-.92	.0017-.0045	.00012-.00035	about .00004
	(2)	.69	.003	.00015	.00004
Large pore 73 \times 44 μ	(1)	.25-.637	.003-.01	.00025-.001	.00004-.00015
	(2)	.45	.005	.00055	.00005
Slot 5 \times 1 mms	(1)	.064-.178	.015-.027	.0014-.006	.00015-.0004
	(2)	.10	.02	.0027	.0002-3

In all curves, Case (1) gives the general range of values obtained; (2) gives a representative experimental value commonly obtained.

All figures refer to the pelvic end of the muscle.

With the fluid slot electrodes α curves are obtained from the pelvic end of the muscle, and β and γ curves appear when the middle region is placed in the slot. The curves obtained with the two pores show an increase in the values obtained with the increased size of the pore, and this corroborates the evidence of Davis that it is the size of the pore which determines the absolute values of the curve.

The effect of denervation. The frogs operated upon were anaesthetised and the main sciatic trunk or its deep posterior branch cut high up the thigh¹. The experiments were performed from 15-44 days after the operation, but only two of 12 experiments were performed less than 30 days after denervation. That denervation had been effected was proved by observing whether the muscle twitched on severance of its nerve during dissection and further by histological examination after staining with methylene blue at the conclusion of the experiment. In general the curves show a decreased excitability to long currents but the "chronaxie" is not appreciably altered. What alteration there is, is observed mainly with the large pore, and, although in some cases the "chronaxie" may be as much as doubled, the general range of values obtained with this electrode is not greatly altered. This slight general decrease of excitability may be attributed to the loss of trophic nerve influences. When fluid electrodes are used, the β and γ curves disappear while the α curve remains practically the same throughout the muscle during the degeneration of the nerve, but may alter when the muscle has been denervated for some time. The values obtained from denervated muscles are given in Table II and a comparison of the curves of normal and denervated muscles may be seen in Fig. 1.

¹ I am indebted to Dr Adrian for operating on all the frogs used in these experiments and my best thanks are due to him for much helpful criticism and advice throughout this work.

TABLE II. Denervated muscles.

Electrode		Threshold (θ) Volts (a)	Time of 1st rise from (θ) Secs. (b)	Chronaxio Secs. (c)	Min. excitation time Secs. (d)
Small pore 3.5 μ dia.	(1)	.575-1.15	.002-.003	.00015-.0003	.00002-.00004
	(2)	.9	.0025	.00018	.00004
	Normal muscle	.69	.003	.00015	.00004
Large pore 73 \times 44 μ	(1)	.487-.75	.01-.025	.00048-.0013	.00008-.00015
	(2)	.50	.012	.001	.0001
	Normal muscle	.45	.005	.00055	.00005
Slot 5 \times 1 mms	(1)	.08-.12	.015-.03	.0021-.005	.0001-.0004
	(2)	.10	.02	.003	.0002
	Normal muscle	.10	.02	.0027	.0002-3

The very small (or absent) change in the "chronaxie" of a muscle after denervation is, at first sight, difficult to reconcile with the fact that, in the body, a denervated muscle needs a "galvanic" current to excite it, instead of a "faradic" (reaction of degeneration), and with the measurements of Bourguignon (6) and Adrian (7), which show a very great increase in the "chronaxie" of human muscle after nerve injury. But the electrodes used for human muscle testing are of large area, and the denervated muscle will therefore give long values comparable to those we have obtained on excised muscles with the slot electrode. When the nerve supply is intact, however, the current presumably excites the nerve fibres in preference to the muscle, and hence the short "chronaxie" of nerve is obtained. Thus, the great increase in the "chronaxie" observed in human subjects after denervation is due, not to any alteration in the time constants of the muscle fibres, but merely to the point of incidence of the stimulus shifting from nerve to muscle, and we should probably be unable to find such a great change if we were able to use an electrode of sufficiently small area on the human subject.

It does not, of course, follow that the "chronaxie" of the muscle remains absolutely unchanged after the degeneration of its nerve. Any change occurring after the nerve has had time to become inexcitable must clearly be due to a change in the time constants of the muscle itself, and such alterations have been reported by Bourguignon (6) and Adrian (7). But these changes are not of the same order of magnitude as the sudden lengthening which occurs in the first fortnight after denervation. This latter change can only be due to the causes mentioned above, *i.e.* to the fact that we are dealing with two distinct excitable tissues which have different time relations when large electrodes are

employed, and not with one tissue whose time relations have been modified by nerve injury.

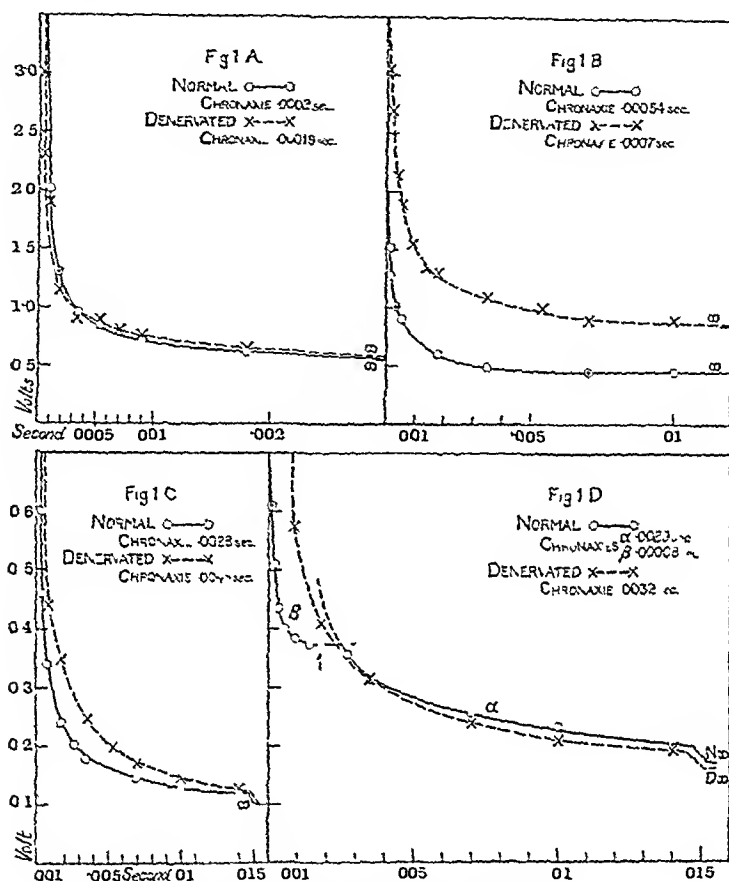


Fig. 1. Normal and Denervated muscles.

- A. Small pore electrode. Pelvic end of muscle.
Temp. Normal 11° Denervated 11.2° C.
Muscle denervated 32 days.
- B. Large Pore electrode. Pelvic end of muscle.
Temp. Normal 9.8° Denervated 11.4° C.
Muscle denervated 22 days.
- C. Slot electrode. Pelvic end of muscle. Simple α curves.
Temp. Normal 10.2° Denervated 11.8° C.
Muscle denervated 37 days.
- D. Slot electrode. Middle region of muscle.
Temp. Normal 15° Denervated 11° C.
Muscle denervated 32 days.

The arrow thus \uparrow on the normal curve indicates the point at which a change in the nature of the contraction was observed. The β curve is not obtained after denervation.

The effect of curari. Doses varying from .5–10 mgms were injected under the skin of the back of the frog, and after $\frac{3}{4}$ hour the frog

was placed in curari-Ringer containing from .01–.3 p.c. curari. The smallest dose used was sufficient to paralyse the muscle, as was shown by failure of stimuli on the nerve, and the absence of any twitch in the muscle when its nerve was severed.

Small doses of curari do not alter the excitability appreciably, any effect being in a contrary direction to that of denervation. The fact that the values given are below those of normal muscles may in part be due to the fact that the temperature of these experiments was higher, and also they were performed at a different period of the year to those on normal muscles. However, Table III and Fig. 2 show that there is a definite increase of excitability to currents of long duration and the curve is hence more rectangular than it is normally. With the larger doses, all the values begin to increase, and in .2 p.c. curari-Ringer the "chronaxie" may be doubled, but yet not outside the normal range.

In three experiments the effect of curari on a denervated muscle was studied, and it was observed that the action was similar to that noted above on normal muscles. This indicates that the degeneration of the nerve fibres does not alter the susceptibility of the actual muscle fibre to curari, and confirms the evidence already obtained that the excitability of the muscle is only affected by relatively strong doses of the drug.

TABLE III. The effect of curari.

Electrode		Threshold (θ) Volts	Time of 1st rise from (θ) Secs.	Chronaxie Secs.	Min. excitation time Secs.
		(a)	(b)	(c)	(d)
Small pore	Weak curari (1)	.35–.85	.0012–.0045	.00007–.000125	About .00001
	(2)	.08	.0025	.00008	.00001
3.5 μ dia.	Stronger „ (1)	.52–1.54	.002–.003	.00008–.000175	About .00001
	(2)	.8	.002	.0001	.00001
Large pore	Weak curari (1)	.187–.375	.002–.02	.00025–.0008	.00005–.00015
	(2)	.27	.012	.00045	.00005
73 \times 44 μ	Stronger „ (1)	.22–.94	.0015–.025	.00025–.0018	.00005–.00015
	(2)	.637	.006	.0007	.0001
Slot 5 \times 1 mm	Weak curari (1)	.091–.187	.012–.04	.0014–.0048	.0001–.0006
	(2)	.118	.035	.0028	.0005
	Stronger „ (1)	.069–.195	.018–.042	.001–.0058	.0001–.0009
	(2)	.145	.03	.0021	.0002

Weak Curari = .5–5 mgms injected under the skin of the back.
.01 % curari-Ringer as bathing fluid.

Stronger „ = 10 mgms injected under the skin of the back.
.02 %–.3 % curari-Ringer as bathing fluid.

It seems evident from these results that the time relations of the excitability of the muscle fibres are not radically changed as a result of denervation or mild curarisation. On the other hand, under these conditions, conduction from nerve to muscle is suspended. This seems

definite evidence against the theory that heterochronism is the main factor which prevents the muscle responding when the nerve is stimulated.

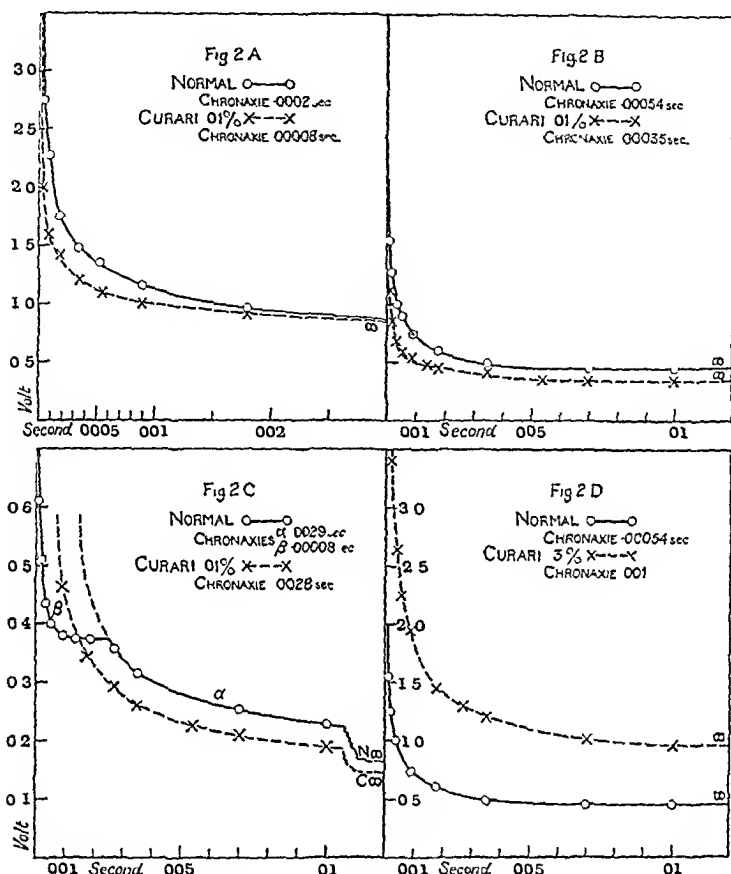


Fig. 2. Normal and Curarised muscle.

- A. Weak curari. Small pore electrode. Pelvic end of muscle.
Temp. Normal 11° Curarised 14.8° C.
Dose. 2 mgms injected. Frog bathed in .01 % curari-Ringer.
- B. Weak curari. Large pore electrode. Pelvic end of muscle.
Temp. Normal 9.8° Curarised 15.2° C.
Dose. 2 mgms injected. Frog bathed in .01 % curari-Ringer.
- C. Weak curari. Slot electrode. Middle region of muscle.
Temp. Normal 15° Curarised 14.6° C.
Dose. .5 mgm injected. Muscle bathed in .01 % curari-Ringer.
Curari abolishes the β curve.
- D. Strong curari. Large pore electrode. Pelvic end of muscle.
Temp. Normal 9.8° Curarised 16.2° C.
Dose. 10 mgms injected. Frog bathed in .3 % curari-Ringer.
Great increase of threshold and chronaxie with this strength of the drug.

For, although the time relations of the muscle may be doubled by curari or denervation, this is by no means a *sine qua non* for failure of conduction

from nerve to muscle. The normal isochronism of nerve and muscle seems to depend upon the technique employed in measuring the excitability of these tissues, and may be present after small doses of curari have abolished the possibility of conduction between them.

Thus, with small doses we obtain the effects described by Lucas on the strength-duration curves, and by Langley⁽¹²⁾ on the action of nicotine, *i.e.* effects which are only obtained in the neural region of the muscle. With larger doses, however, the muscle itself is affected, as previous observers have noted, but it is not definitely known whether this action is due to the drug itself or to impurities, *e.g.* salts. This action is, however, subsidiary, and not the main factor involved in the failure of conduction between nerve and muscle. It follows that if curari does not act on the muscle substance to produce paralysis it must act upon something which is distinct from both these substances, and this agrees with the classical theory as to the mode of action of drugs on striated muscle. The intermediate substance may be comparable to the nerve endings which Claude Bernard supposed were the seat of action of curari, or to the neuro-muscular junction postulated as the result of the experiments of Langley⁽⁸⁾, Anderson⁽⁹⁾, Brodie and Dixon⁽¹⁰⁾, and Elliot⁽¹¹⁾. However, Langley, from later observations⁽¹²⁾ upon the action of nicotine on striated muscle, developed the theory of a receptive substance situated on the muscle side of the nerve endings and it is generally supposed that the β curve of Lucas represents the time relations of this substance. But whereas Langley found that denervation did not abolish the action of nicotine, as regards tonic contraction, the β curve of Lucas has not been found in a muscle after denervation.

There is little evidence, however, as to the nature of this substance, but the work of Davis suggests that it may have an extremely small structure. Davis used capillary pore electrodes of different sizes and found that the smaller the pore used, if less than about 75μ in diameter, the smaller is the "chronaxie" obtained. Hence when the electrode is of small area relative to the tissue element stimulated, it is the size of the electrode and not the tissue that determines the absolute values obtained.

Jinnaka and Azuma⁽⁵⁾ using a 10μ pore obtained from the non-neural region of a sartorius muscle a curve similar to the β curve of Lucas with a "chronaxie" of about $\cdot 0003$ sec. From what has been said above, however, this does not imply that a substance having these time relations lay under their electrode but rather that the area of stimulation

employed was such as to give these values when applied to the individual muscle fibre. On the other hand, my experiments with the two fluid slot electrodes of different sizes show that when a relatively large electrode is used any alteration in its size (provided it is large enough to stimulate the whole muscle) does not affect the values of the time constants obtained. When, therefore, a curve is obtained with this type of electrode, such as Lucas' β curve which differs radically from the normal α curve, it may be accepted as definite evidence of a distinct excitable substance in the region of the muscle which is being stimulated, *i.e.* the neural region. This conclusion as to the definite existence of an intermediate substance which is the seat of action of drugs, etc., harmonises with the phenomena of decremental conduction, Wedensky inhibition, fatigue, etc., already observed by various authors and explained by them as due to the existence of such a substance between nerve and muscle.

SUMMARY.

The work of H. Davis has shown that the size of the stimulating electrode relative to that of the tissue elements plays a great part in determining the excitability constants, *i.e.* "chronaxie" and the strength-duration curve, of that tissue. The changes in "chronaxie" following denervation and curarisation have been investigated in the light of Davis' work.

When a muscle is denervated it is found that the "chronaxie" of the muscle fibres themselves does not alter to any great extent, and the lengthening of the "chronaxie" observed is due to the point of effect of the stimulus passing from nerve to muscle.

In the case of curari, it is concluded that the effect of the drug in paralysing the muscle is not due to any action on the muscle fibres, though such an action may be observed when larger doses are employed.

The bearing of these results on the mechanism of conduction of the impulse from nerve to muscle is briefly discussed.

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THE INFLUENCE OF THE BLOOD SUPPLY ON PANCREATIC SECRETION. By B. P. BABKIN.

(From the Physiological Institute, University College, London.)

THE importance of the normal blood supply of a gland for its activity is not of the same degree for all kinds of digestive glands. In the salivary glands the blood supply can be decreased to a certain degree without great damage to their secretory activity (Heidenhain(1), Langley and Fletcher(2), Carlson, Greer and Becht(3), Gesell(4)). The pancreatic gland seems to be extremely sensitive to the changes in the circulation provoked by stimulation of different nerves (Bernstein(5), Pavlov(6), Gottlieb(7), Mett(8), May(9), Edmunds(10), Anrep(11)), or by substances with vasoconstrictor character (Gottlieb(7), Benedicenti(12), Edmunds(10)), or by compression of the aorta above the coeliac artery (Pavlov(6), May(9), Anrep(11)). On the other hand, the pancreatic gland continues to secrete some time after the blood pressure falls to zero (Pavlov(6), May(9)). It must be added that the more copious the pancreatic secretion the more difficult it is to inhibit it.

Thus some points concerning the interrelations of the secretion and blood supply in the pancreatic gland are not quite clear. It seems that not every interference with the circulation influences the secretory work of the gland, but only interference of special kinds. The following observation of Heidenhain and one of the experiments of Gottlieb lead one to accept this supposition. Heidenhain(14) observed in a dog marked periodic variations in the blood-pressure. With the rise in the blood-pressure came a lessening of the pancreatic secretion, with its fall occurred an increase of the secretion. Gottlieb(7) inhibited the spontaneous pancreatic secretion in a rabbit with strychnine. The injection into the blood of chloral hydrate, which relaxed the blood-vessels contracted by strychnine, increased it again. Since chloral hydrate not only paralyses the vaso-constrictor centre, but stimulates the pancreatic secretion also (Wertheimer et Lepage(15)), Gottlieb's interpretation of the phenomenon observed cannot be accepted without further investigations.

All this led me to reinvestigate the question. My task was greatly facilitated because in chloralose I found a narcotic which itself produced

a moderate secretion of the pancreatic juice and did not affect the action of hormones nor of secretory nerves.

Methods. The experiments were performed on dogs. The day before an experiment the dog had his last meal not later than 5 p.m. Water was left during the night. The next morning the stomach as a rule was empty; it contained only alkaline or weakly acid mucous. For a short while the animal was narcotised with chloroform and ether. Chloralose (0.1 g. per kilo weight) was then injected into a vein. A longitudinal incision was made in the pylorus and the stomach was separated from the duodenum by means of circular suture which passed only through the mucous and submucous membrane in the distal part of the pylorus. A cannula was introduced into the main pancreatic duct and was connected with the tubing on a scale each division of which corresponded to .005 c.c. After establishment of artificial respiration the chest was opened. Both vagi were tied and cut in the neck and below the heart, the right splanchnic nerve also. (If there are not special indications in the description of the experiments it means that the vagi were cut in the neck and below the heart.) A string was put round the left splanchnic nerve in the abdomen. Later by strong quick pulling of the two ends of the string the nerve could be torn in two, without opening the abdomen. In most of the experiments the blood-pressure was registered. (In all experiments quoted below the average blood-pressure is indicated for a given time. If two or more figures appear they represent the consecutive oscillation of the blood-pressure.) At the end of the experiment the contents of the stomach were examined. In no case was accumulation of gastric juice noticed.

Secretory action of chloralose. Chloralose (synonyms = glucochloral, anhydrochloral, α -chloralose) is obtained by means of heating anhydrous chloral hydrate and dry glucose in a closed tube at 100° C. for one hour (16). It retains some of the properties of chloral hydrate and among others the property of stimulating the pancreatic secretion. The following experiment on a dog under chloralose with splanchnic nerves intact shows that the pancreatic secretion lasted 2 hours 15 mins. The oscillations of the secretion and its interruption resulted from the special experimental interventions.

Exp. 1. The secretion is noted in 5 minute intervals.

3, 2, 4, 3, 4, 3, 3, 2, 2, 3, 1½*, 1½, 1, 2, 3, 2, 3, 3, 2½, 3½, 3†, 2, 1, 2, 2, 3.

* Rhythmic stimulation of the peripheral end of the right vagus in the neck during 5 mins. Coil 7 cm.

† Right sympathetic nerve cut in the chest.

The next experiment shows more distinctly the secretory action of chloralose and the action of secretory nerves under it. In this special case the spinal cord in a dog was cut below the medulla and the brain destroyed.

Exp. 2. Spinal dog. 6 kgm. Secretion is noted every 5 mins.

1, 1, 5*, 7, 3, 2, 2 (0.22 chloralose into the vein), 3, 6, 7, 5 (again 0.22 g. chloralose), 5, 14, 9, 6, 4, 3, 4, 10*, 30, 9, 4, 2, 2.

* Stimulation of vagi below the heart during 5 mins., each nerve stimulated 1 min. Coil 7.5 cm.

Like moderate doses of other narcotics (chloroform, ether, morphia) chloralose does not disturb the secretion provoked by the hydrochloric acid or secretin. It is not only not antagonistic to pilocarpine but a summation of the action of these two drugs can be observed.

As to the composition of the pancreatic juice secreted under the influence of chloralose it approaches the composition of the "nervous" juice. Thus the lowest figures obtained were: solids 3.97 p.c.; organic substances 3.20 p.c.; ash 0.77 p.c. 1.25 c.c. of juice were secreted during 1 hour 25 mins.

All this shows that chloralose is a very suitable narcotic for the study of the pancreatic secretion. This anæsthetic gave the opportunity to investigate more closely the interrelations between the secretory process and the blood supply of the pancreatic gland. The pancreatic secretion was provoked not only by secretin, viz. hydrochloric acid, but also by stimulation of the secretory nerves.

The influence of section of the splanchnic nerves on the pancreatic secretion provoked by secretin. The deprivation of the pancreatic gland of sympathetic innervation, which coincides with the fall of the blood-pressure greatly increases the secretion provoked by secretin.

Exp. 3. Dog under chloralose. Steady inflow of secretin (1 cc. in 1 min.) through the jugular vein. Secretion noted every 2 mins.

P.J.	22	25*	22†	38	58	53	55
B.P.	40	44-30	42-25	23	25	24	25

* Right sympathetic torn in two in the chest.

† Left splanchnic torn in two in the abdomen. The rate of the heart's beat increased from 244 per min. to 260.

After the section of the splanchnic nerve, the same dose of secretin provokes nearly a double quantity of pancreatic juice in the same time. This is seen from Exp. 4.

Exp. 4. Both vagi cut in the neck and below the heart and right splanchnic in the chest. Secretion noted every 5 mins.

(A) 4.17 p.m.

P.J.	0	62*	55	17	=134 div. in 15 mins.
B.P.	104	51-73-93	100-104	106	

4.37 p.m. Left splanchnic cut in the abdomen.

(B) 4.45 p.m.

P.J.	1	146*	85	21	4	=252 div. in 15 mins.
B.P.	60	34-58-61	64	—	65	

(C) 5.07 p.m.

P.J.	0	157*	97	29	5	=278 div. in 15 mins.
B.P.	65	43-66-64	67	69	73	

* At the beginning of these 5 minute intervals 1 c.c. of secretin introduced into the jugular vein.

Thus the section of the splanchnic nerves markedly increases the secretion of pancreatic juice provoked by secretin. It coincides with a more or less pronounced fall of the systemic blood-pressure. The same relation has been seen in cases of pancreatic secretion provoked by chloralose.

The most simple explanation of the phenomena observed is that the abdominal viscera, and in particular the pancreatic gland, as a result of elimination of the sympathetic innervation, receive much more blood. And this creates very favourable conditions for the secretory work of the gland. These experiments do not support the view that in the sympathetic nerves there are special inhibitory fibres to the secreting cells of the pancreatic gland. But they by no means deny this. No doubt an assertion that every inhibition of pancreatic secretion is of vasomotor origin is not quite accurate. Anrep(11) and Korovitzky(17) have described the part played by the pancreatic ducts in the inhibition of the secretion. But further proofs are necessary to substantiate the theory that the sympathetics or vagi carry nerve fibres to the pancreatic gland which are capable of making the secretory cells refractory to the nervous or humoral impulses or of blocking the already elaborated secretion in the cells themselves.

Two series of experiments were performed for the more precise determination of the influence of vascular activity of the pancreatic gland: (1) with the temporary lowering of the blood-pressure, and (2) with the rise of it.

Influence of the lowering of the blood-pressure upon the pancreatic secretion. Two methods were employed: (1) rhythmic stimulation of the peripheral ends of the vagi in the neck, the nerves being cut under the heart; (2) compression of the inferior vena cava in the chest. In both

cases the secretion of pancreatic juice was provoked by secretin, hydrochloric acid or chloralose.

The stimulation of the peripheral end of the vagus in the neck, followed by a sharp fall of the blood-pressure and slowing of the heart, does not affect the pancreatic secretion. But if after the stimulation is finished the blood-pressure goes up a marked slowing of the secretion is observed. This can be seen in Exp. 5. The secretion of the pancreatic juice was provoked by means of a steady flow of secretin into the jugular vein (1 c.c. every min.).

Exp. 5. The secretion is noted every 2 mins.

(A) P.J.	24	23	25*	11	16	19	19
B.P.	70	73	52	74-100	118-96	86	86
(B) P.J.	12	12	12†	10	14	16	13
B.P.	89	96	53	76-100	86	86	90

* Rhythmic stimulation of vagi in the neck. Coil 7 cm. † Ditto. Coil 0½ cm.

The rise of the blood-pressure following the first stimulation of the vagi (A) was bigger and lasted longer than the second (B). Corresponding with this, the inhibition of the secretion in the first case was more pronounced than in the second. The most striking feature of this experiment is the absence of any inhibition whatever of the activity of the gland during the actual stimulation of the vagi. And this occurred in spite of a notable average fall of blood-pressure and slowing of the pulse rate from 132 or 133 beats per min. to 30 or 36 beats.

In Exp. 6, A shows very distinctly that no changes occur in the secretion if the blood-pressure is not affected after the stimulation of the vagi.

Exp. 6. A. Splanchnics intact. Constant flow of secretin into the jugular vein (0.5 c.c. every min.). Flow of pancreatic juice per 1 min.

P.J.	40	32	36	32	36	38*	33*	39*	37	43	36	38
B.P.	98	103	104	97	84	48	52	50	87	80	81	78

* Rhythmic stimulation of vagi in the neck. Coil 6½ cm.

The same dependence of pancreatic secretion on the rise of a probable constriction of the vessels of the gland, can be seen when other secretory stimuli are employed, *i.e.* hydrochloric acid, chloralose, etc. Thus in Exp. 6, B, the great fall of blood-pressure produced by stimulation of vagi in the neck did not disturb the typical rise of the secretion under hydrochloric acid. But after the stimulation the secretion was inhibited coinciding with the rise of the blood-pressure (Fig. 1).

Exp. 6. B. Both splanchnics intact. 25 c.c. of 0.4% HCl introduced into the duodenum. Flow of the pancreatic juice every 2 mins.

P.J.	0	9	18	18	27*	10	13	11	6	6
B.P.	91	102	116	106	58	124-150	140-122	112	108	94

* Vagus stimulated in the neck. Coil $6\frac{1}{2}$ cm.

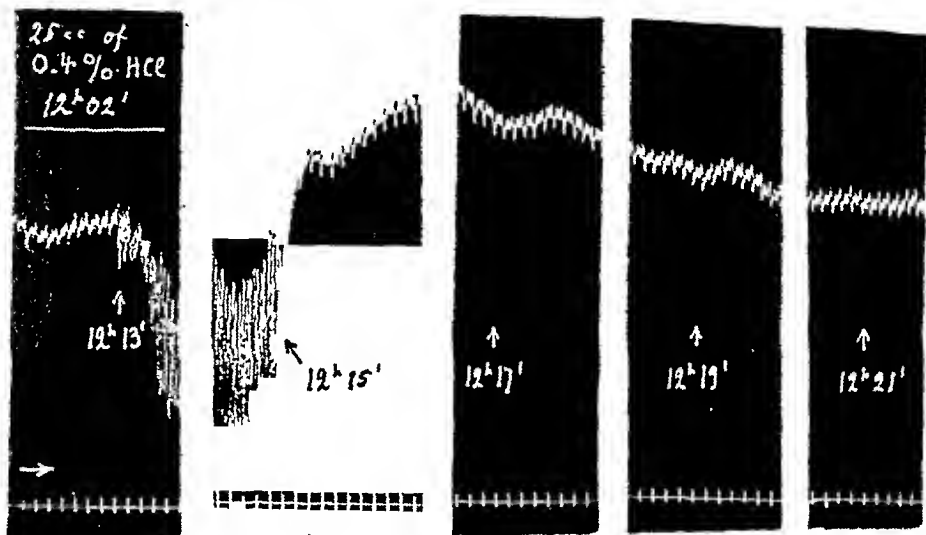


Fig. 1.

It must be added to this that in some cases a certain inhibition of chloralose secretion was already observed during the stimulation of the vagi (cf. Exp. 1). This phenomenon is not peculiar to the chloralose secretion. It can be observed during the action of other stimuli, if only the secretion is slow enough.

The cause of the rise of blood-pressure probably is, as Anrep and Starling (unpublished work) have shown in experiments with crossed circulation, the stimulation of the vaso-motor centre in the brain, provoked by the fall of blood-pressure. And indeed after the section of both splanchnics this rise of the blood-pressure is very small or even absent. Corresponding to this the inhibition following the stimulation of the vagi is insignificant or absent (Exp. 7, Fig. 2).

Exp. 7. Both splanchnics cut. A. First injection of 2 c.c. of secretin into the jugular vein at 4.44 p.m. B. The second injection of 2 c.c. of secretin at 5 p.m. Flow of pancreatic juice recorded every 2 mins.

(A) P.J.	13	33	14	10	5	3	1	=79 div. in 14 mins.
(B) P.J.	2	32	23*	18	4	3	1	=83 div. in 14 mins.
B.P.	23	24	16	23	21	20	18	

* Rhythmic stimulation of vagi during 2 mins. Coil 7 cm.

In order to produce a more considerable congestion of blood in the abdominal viscera periodic closing and opening of the vena cava inferior

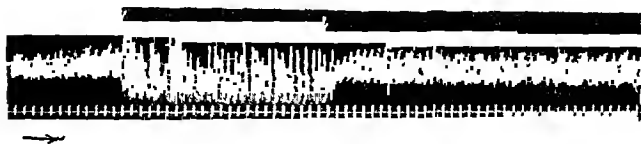


Fig. 2 Vagi stimulated in the neck (between the arrows), coil at 7 cm.

above the diaphragm during 2 mms. was performed. No marked changes in the flow of pancreatic juice could be observed during this procedure. The usual inhibition of the secretion was noted only if a rise of blood-pressure occurred. This can be shown from Exps 8 and 9 (Fig 3).

Exp 8 Both splanchnics cut. 1.2 c c secretin introduced into the jugular vein. Flow of pancreatic juice and average blood pressure noted every minute

P J.	2	31	37	30	34	11	27	20	16	19	13	7	7	3	3
B P.	65	67	69	v c	compr.	80	75	74	72	74	72	76	76	81	—

The gradual rise of the blood pressure towards the end of the observations depends upon the expiration of the action of the secretin

Exp 9. Vagi and spl cut. Continued flow of secretin into the jugular vein per 1 c c every min. Two minute intervals for recording the secretion

P J.	49	53	48	48	41	43	46	44	44	45
B P.	23	23	24 v c compr 32			29 v c compr 31			31	30

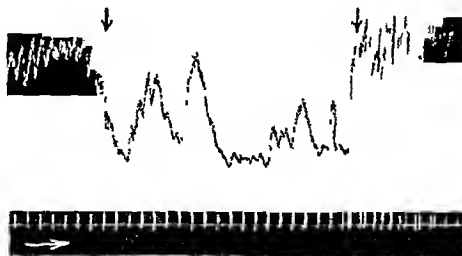


Fig 3 Vena cava compressed (between the arrows)

The insignificance of the rise of blood-pressure in the experiments depends upon the exclusion from the influence of the vaso-motor centre of a vast vascular district belonging to the abdominal viscera.

ON THE LOCATION AND NATURE OF THE ACTION OF INSULIN. BY J. H. BURN AND H. H. DALE.

(From the National Institute for Medical Research, London.)

THE phenomenon of the disappearance of dextrose from the blood under the influence of injected insulin is now familiar, but the method of the disappearance remains obscure. A full review of the relevant literature has recently been published by Lesser (1924). Suggestions have been made of a change of the dextrose into a reactive form (Winter and Smith)¹, in which it becomes liable to metabolic attack; but whatever the future may decide as to the validity of this conception, it leaves certain broader issues undetermined. The evidence as to the further fate of the sugar is meagre and to some extent conflicting. The appearance of glycogen in the liver of the diabetic animal when insulin is given (Banting and others) suggests a synthesis into this substance; on the other hand, it has been established that the glycogen disappears from the liver and the muscles of a normal animal, when an excessive dose of insulin depletes the blood of sugar (Dudley and Marrian). Again, the rise of the respiratory quotient, produced by insulin in a diabetic animal (Banting and others), suggests that some of the sugar is removed by combustion; on the other hand, we have the evidence of a great depression of respiratory metabolism in normal animals poisoned with insulin (Dudley and others). Nor is the evidence clearer as to the location of the effect, whatever its nature may be. Insulin will not act on the sugar of the blood outside the body, so that some organs are concerned in its effect. Neither the brain (Olmsted and Logan) nor the liver (Mann and Magath) is essential to the action; but with regard to what remains the evidence is incomplete, and not altogether consistent. Hepburn and Latchford showed accelerated disappearance of glucose from Locke's solution, perfusing an isolated rabbit's heart, when insulin was added, but their evidence left open the question whether the extra glucose was removed by combustion or synthetic storage. Macleod mentions unsuccessful attempts to investigate the participation of skeletal muscles by adding insulin to blood artificially perfused through limbs, and states that analysis of arterial and venous blood, from the

¹ For references, see end of paper.

limbs of animals under insulin, showed no increased discrepancy between the content of sugar in the blood before and after circulating through the muscles, even in diabetic animals. More recently evidence that insulin increases the loss of blood-sugar observable in passage through a limb has been recorded by Cori, Cori and Goltz, working on rabbits, and by Lawrence working on a human diabetic.

The experiments described in this paper were undertaken with the view of providing additional evidence on two relatively simple and primary points: (1) whether the sugar which disappears under insulin is immediately oxidised, or stored in some form which has hitherto escaped detection; and (2) what organs are concerned in the removal, whether by destruction or synthesis. There are two series of experiments—those on the isolated mammalian heart, and those on the decapitated and eviscerated cat. They will be separately described.

PART I. EXPERIMENTS ON THE ISOLATED MAMMALIAN HEART.

Methods. The main object of the experiments was to confirm and extend Hepburn and Latchford's results with the particular object of obtaining evidence as to whether the additional glucose removed under insulin is used immediately as a source of energy, or stored in the heart in some form. Like Hepburn and Latchford we used the apparatus of Locke and Rosenheim, but modified it in certain details. One object we had in view was to measure, in some experiments, the rate of removal of glucose by the same heart, when perfused with or without insulin. This involved two periods of perfusion—a preliminary control period, and a second period with insulin. With the scheme used by Locke and Rosenheim, and adopted by Hepburn and Latchford, in which about 250 c.c. of fluid are circulated, a perfusion of several hours is required in order to obtain such a diminution of sugar percentage as can be measured with reasonable accuracy. Even during the latter part of one such period, as Hepburn and Latchford point out, the heart-beat becomes notably less powerful, and the normal disappearance of sugar correspondingly slow. To subject the heart to two such periods in succession would require a long working day for each experiment, while the test of the effect of insulin would be complicated by applying it to a heart already well past its best vigour. Reduction of the volume of the circulating fluid from 250 c.c. to, say, 50 c.c. would, at the same rate of removal, give the same reduction of sugar percentage in one-fifth of the time, so that a complete experiment could be made, with control and insulin periods, within the range of the heart's optimum activity.

was repeated, with a fresh set of absorption tubes, when a second period of perfusion was given to the same heart.

The percentage of dextrose in the perfusion fluid at the beginning of the experiment, or of each period thereof, was in the neighbourhood of 0.2 p.c., the figure taken being not that of the proportion originally added to the solution, but that found by analysis at the beginning of the observation period. A proportion above that of the normal blood sugar was used deliberately, so that, in the event of a large disappearance under the influence of insulin, the magnitude of the effect might not be masked by causing the heart to beat for a large part of the time on a sugar percentage below the physiological limit.

The insulin used was a sample of "insulin hydrochloride" prepared by our colleague, Dr Dudley. The sample was of moderate activity, a "physiological unit" (convulsant dose for a 2 kilogram rabbit) being contained in about 0.5 mgm. The insulin was dissolved in a small volume of the Locke's solution, and this solution was added to that used directly for perfusion, or for admixture with the animal's blood, in such proportions as to make final concentrations of 1 part of the insulin in 500,000 to 250,000 of the perfusion fluid. We ascertained that no change in reaction, as determined by delicate indicators, was caused by this addition. If it were assumed that the whole of a dose of insulin injected into an animal entered and remained in the circulating blood, then, since a rabbit of 2 kilos. has about 125 c.c. of blood, the proportion of 1 in 250,000 (0.5 mgm. in 125 c.c.) might be taken to represent a "convulsant" concentration of this sample of insulin. The assumption is, in fact, not warranted, the concentration used being probably much in excess of that occurring in the circulation of an animal receiving a single convulsant dose.

Normal hearts perfused with Locke's solution. In all the successful experiments of this class the heart was given only one period of perfusion, either with or without insulin, the latter, when present, being in the proportion of 1 part, of the sample of hydrochloride used, in 500,000 parts of the Locke's solution. When the freshly isolated heart was perfused with such a mixture we obtained a good coronary flow and a rapid, vigorous beat. It will be seen, indeed, that it was usually more rapid than in the normal heart. The results are given in Tables I and II. The tables include all the results obtained in experiments of this class on rabbits' hearts, with the exception of one of the control hearts, in which a good beat was never established, and one of the insulin hearts, in which a bubble of gas was subsequently found in a branch of

the coronary artery. In all the tables the figures for dextrose and CO_2 represent the amounts disappearing from the perfused solution, and collected in the absorption tubes respectively, in milligrams per gram of heart per hour. By the "percentage of yield" of CO_2 , we mean the amount actually collected, expressed as a percentage of that which would be produced by complete combustion of the disappearing dextrose. Rate is given in beats per minute, the maximum and minimum attained during the period of observation being recorded.

TABLE I Rabbits hearts No insulin

	Dextrose	CO_2	% yield of CO_2	Rate	Weight of heart
1	1.77	3.35	129	142-176	4.2
2	1.76	2.83	109.6	111-136	5.9
3	1.82	2.16	81	110-136	4.8
4 a	1.14	3.42	204.5	136-158	4.6
4 b	1.15	4.05	240	120-136	—
5	0.54	2.91	367.5	128-141	6.3
Average	1.36	3.12			

Table I, 4 a and b, show the results of two successive periods of perfusion of the same heart, each of two hours, with Locke's solution. The dextrose fell in the first period from 0.226 to 0.205 p.c. and in the second from 0.205 to 0.182 p.c. It will be seen that in all cases except No. 3 the CO_2 collected is in excess, and in two experiments very greatly in excess of what would be produced by complete combustion of the dextrose lost from the perfusion fluid. The heart, therefore, under these conditions is supplying part of its energy needs, and sometimes the greater part, by combustion of its own stored material, in preference to the excess of dextrose offered in the Locke's solution.

TABLE II Rabbits hearts Insulin 1 in 500 000

	Dextrose	CO_2	% yield of CO_2	Rate	Weight of heart
6	3.35	4.79	97.5	180-200	4.8
7	3.67	5.86	109	166-180	4.6
8	2.64	2.62	67.6	110-136	6.2
9	3.59	4.16	79	176-200	4.9
10	3.94	5.87	101.5	200-214	3.8
Average	3.44	4.66			

There are several points to be noted in Table II. In the first place it will be seen that our results confirm those of Hepburn and Latchford as to the greater rate at which dextrose disappears from the perfusion fluid when insulin is present, than in its absence. The lack of clear indication as to dosage in their paper makes it impossible to compare

our results quantitatively with theirs. Our figures, however, though not showing so large an acceleration as theirs, show a very pronounced difference, the dextrose disappearing more than two and a half times as fast, on the average, with insulin than without it. The significance of this difference is slightly weakened by the fact that the hearts receiving insulin showed, on the whole, a more rapid and vigorous beat and a better sustained activity than those on plain Locke's solution. The difference is not conspicuous enough to account for so great an increase in the rate at which dextrose disappears, and cannot at all account for the failure of the CO_2 output to show a proportionate increase. It will be seen that, although the average CO_2 output is greater with insulin than without it, the difference is not nearly so great as with the dextrose. The figures for "percentage yield" show that in only one of the experiments with insulin was the CO_2 produced distinctly greater than that which the oxidation of the lost dextrose could have yielded; in the other four cases it was either about 100 p.c., or definitely less. We may leave discussion of the point until we have presented the figures obtained with hearts perfused with mixtures of blood and Locke's solution.

Normal hearts perfused with diluted blood. The animals' own blood was used, diluted, after whipping and filtering, with about an equal quantity of Locke's solution. The latter was made up with 0.3 p.c. of dextrose, with the aim of producing a blood-mixture with a sugar percentage in the neighbourhood of 0.2 p.c. In most of the experiments only one period of two hours' perfusion was given, either with or without insulin, but in a few cases control and insulin periods were given in succession to the same heart. Tables III and IV give the results of the single-period experiments.

TABLE III. Normal hearts No insulin.

	Animal	Dextrose	CO_2	% yield of CO_2	Rate	Weight
11	Rabbit	2.48	4.78	131.4	110-150	4.9
12	"	4.56	8.30	124.1	210-250	2.6
13	"	3.20	6.18	131.6	188-208	3.0
14	"	1.60	5.50	234.4	170-214	2.7
	Average	2.96	6.19			
15	Cat	1.9	3.38	121	130-192	5.8
16	"	3.7	4.06	74.8	170-234	6.1
	Average	2.8	3.72			

There is, again, a clear indication of an increased rate of disappearance of dextrose from the perfusion fluid when insulin is present. The contrast is not quite so pronounced as in the experiments with Locke's solution.

TABLE IV Normal rabbits' hearts Insulin 1 in 250 000

	Dextrose	CO ₂	% yield of CO ₂	Rate	Weight
17	5.75	7.16	85	176-194	4.5
18	4.20	6.17	100	188-230	3.0
19	5.0	6.06	82.6	184-204	2.7
20	5.62	5.90	71	214-250	4.4
Average	5.14	6.32			

It is associated, again, with some increase of the rate of the beat, but this is obviously inadequate to account for the whole of the difference in the dextrose figures. The difference in the figures relating CO₂ production to loss of dextrose is, in this case also, even more significant. If we confine our attention to the figures for rabbits' hearts, which are strictly comparable, it will be seen that in Exp. 12, in which the sugar-loss without insulin, 4.56, is unusually high, this is associated with the highest output of CO₂ seen in the whole series, 8.3, giving a percentage CO₂ yield of 124. If we take from the insulin series the case with a practically identical range of heart rates, Exp. 20, we find a higher rate of sugar-loss, 5.6, with a much lower rate of CO₂ production, 5.9, giving a percentage yield of only 71. Only in one of the experiments without insulin, No. 16 on a cat's heart, is the percentage yield not in excess of 100, while in all the insulin experiments it is 100 or less.

This change in the proportion between CO₂ production and sugar disappearance is shown in an even more convincing manner by two experiments (Table V) in which the heart was perfused for a first period of two hours with a mixture of blood and Loëke's solution, and during a second two hour period with an equal quantity of the same mixture with the addition of 1 in 250,000 insulin hydrochloride.

TABLE V Rabbits' hearts Normal and insulin periods

		Dextrose	CO ₂	% yield of CO ₂	Rate	Weight
21	(a) No insulin	4.23	5.34	86	162-200	3.5
	(b) Insulin	5.64	4.55	55	136-172	—
22	(a) No insulin	3.94	5.16	88	154-187	5.0
	(b) Insulin	3.03	2.45	55	96-146	—

The notes record that the heart beat, in both cases, not only more slowly but less powerfully in the insulin period than in the control. The coronary flow was also much reduced, in comparison with that in the first periods, especially in Exp. 22. Nevertheless, the rate of sugar disappearance in 21 *b* shows a definite excess over that in 21 *a*, while the CO₂ output is as definitely reduced. In 22 *b* the rate and vigour of

the beat are so much less under insulin that the figure for dextrose shows a fall from that in 22 *a*; but the CO_2 output is reduced so much more in proportion that the figures for percentage yields of CO_2 , in the corresponding periods of the two experiments, are strikingly similar. Reference to Table I, Exp. 4 *a* and *b*, will show that there is no similar reduction in the percentage yield of CO_2 , when a second period of perfusion is given without insulin.

Hearts from diabetic cats. From a series of young cats, one of us (H. H. D.) removed the pancreas by aseptic operation under ether. The operation in this species is a relatively easy and rapid one, not more than two ligatures for blood vessels being required. The cats recovered quickly, and usually took food greedily a few hours after the operation. The first sample of urine obtained after the operation always contained abundant dextrose, and by the following day a severe diabetes had set in. Even in the absence of kinking of the duodenum, which is liable to occur owing to the gap inevitably made in its mesentery, the cats became drowsy and weak by the third or fourth day, though the wound and peritoneum were healthy. At this stage they were killed, and in three cases the hearts were perfused, in one case with pure Locke's solution, in the others with mixtures of the cat's own blood and Locke's solution in equal parts. In each of these experiments, two periods of perfusion were given, the first without, the second with insulin added to the perfusion fluid. The results are shown in Table VI. Exp. 23 was carried out with Locke's solution containing 0.19 p.c. of dextrose, and in the second period 1 in 500,000 insulin, and Exps. 24 and 25 with blood-Locke mixtures containing initially 0.28 and 0.13 p.c. of dextrose respectively, and in the second periods 1 in 250,000 insulin.

TABLE VI. Hearts of diabetic cats.

		Dextrose	CO_2	% yield of CO_2	Rate	Total beats	Weight
23	(a) No insulin	0.83	2.14	176	124-152	—	10.5
	(b) Insulin	1.14	1.74	104	84-127	—	—
24	(a) No insulin	1.4	2.94	143	100-180	11,044	8.1
	(b) Insulin	2.51	3.2	87	118-166	14,904	—
25	(a) No insulin	2.46	4.1	113	172-262	24,850	5.9
	(b) Insulin	3.54	5.03	97	150-240	23,460	—

Perhaps the first thing to be noticed about the figures in Table VI, is their remarkable similarity to those obtained with the normal hearts. The dextrose consumption in the control period is, in each case, below the average for normal hearts, but it is not in any case clearly outside the range of the figures obtained from normal hearts perfused with a similar fluid. Insulin, in every case, causes an increased rate of dextrose

consumption, but this is not more obvious than with the normal hearts. The results are, in that respect, in strong contrast to those obtained by Maclean and Smedley, who, with hearts from diabetic dogs, observed very little or no disappearance of dextrose when pancreatic extract was not added to the Locke's solution. Our observations do not suggest that there is any fundamental change in the metabolism of the heart muscle, produced by complete removal of the pancreas and the ensuing diabetes. That the dextrose consumption, both with and without insulin, is lower than that of most normal hearts under comparable conditions, is quite adequately explained by the poor condition of the heart muscle, produced by the progressive toxæmia which had set in before the animals were killed for the experiment.

It will be noted, again, that, as with the normal hearts, the output of CO_2 may be either diminished or somewhat increased in the insulin period, but that, in any case, it is not increased proportionately to the consumption of dextrose, the percentage yield of CO_2 being well above 100 in all the normal periods, and below 100 in two out of the three insulin periods. In Exps 24 and 25, we counted the rate of the beat every five minutes during both periods, so that we could calculate with reasonable accuracy the total number of beats for each period. It was not possible actually to measure the work done per beat, but it may be stated that inspection did not suggest any increase in amplitude, but rather a diminution. If we assume that the work per beat was unchanged, the total work for the two hour period under insulin would, in Exp 24 be 15/11 of that in the control period. If the dextrose figure showed a corresponding increase, it would rise from 1.4 only to $1.4 \times 15/11 = 1.9$, whereas it actually rises to 2.5. In Exp 25, the discrepancy is more obvious, since there is a 44 per cent increase in the rate of disappearance of dextrose, with a small decrease in the total number of heart beats.

Effect of glycolysis by blood alone. One complication of these experiments, in which diluted blood was used for perfusion, remains to be mentioned, namely the loss of dextrose due to the glycolytic action of the blood itself. The effect is a relatively small, but a variable one, so that we did not feel justified in applying a constant correction. In one experiment, we divided the blood saline mixture into two portions, and kept one in the cold room while the first was circulated slowly through the apparatus, at 37°C , without a heart, for two hours, the procedure being then repeated with the second portion. In this case the percentage of dextrose in the first sample fell, during two hours' warming, oxygenation and circulation, from 0.232 to 0.205 per cent, in the second sample, with

identical treatment, from 0.218 to 0.206 p.c. The true figure for dextrose consumption, by a heart perfused with this mixture, would therefore be obtained by subtracting the final percentage not from the observed initial percentage, but from 0.206 p.c. In another experiment we investigated the amount of the correction which would thus be introduced. The blood-saline mixture was circulated by itself for two hours, while the heart was preserved at about 0° C. A sample of the blood mixture was then removed for analysis, and the remainder circulated for a further two hours through the beating heart, which weighed 7 grams. During the first period, without the heart, the dextrose fell from 0.233 to 0.215 p.c. During this second period, with the heart, it fell from 0.215 to 0.108 p.c. The gross disappearance of dextrose during the second period, with 35 c.c. of fluid in circulation, amounts to about 2.7 mgm. per gram of heart per hour. If we assume that glycolysis, by blood alone, continues at the same rate in the second as in the first period, we get a nett removal of dextrose by the heart at the rate of 2.2 mgm. per gram per hour. The correction is, therefore, not a large one in relation to the accuracy of the method as a whole, and it should be noted that the correction would have less effect as the total disappearance became larger with insulin. We confirmed, under the conditions of circulation and oxygenation here used, the observation of Eadie, Macleod and Noble that the addition of insulin to blood alone does not affect the rate of glycolysis. The question may be raised as to whether a glycolysis by blood alone could account for the removal of dextrose which we observed with the hearts from diabetic cats. It may be stated that, on the one hand, the maximum simple glycolysis which we observed would account for only a part of the sugar disappearing in the perfusion of such hearts by blood mixtures, and, on the other hand, that in the one experiment which we made to observe the rate of glycolysis by blood from a diabetic cat, we could detect no loss of sugar in two hours' circulation. We do not advance this as evidence of loss of glycolytic power by the blood of the depancreatized animal, glycolytic action by shed blood being, in any case, a very variable phenomenon; but it is at least fair to assume that no larger correction ought to be applied to the experiments on hearts from diabetic animals than to those on normal ones. It is further to be noted that the heart from a diabetic cat, perfused with Locke's solution alone, gave a figure for disappearance of dextrose within the range of those obtained from normal hearts under the same conditions.

DISCUSSION OF THE ABOVE RESULTS

The impossibility of measuring the use of oxygen with the Locke scheme of circulation, so that the respiratory quotient cannot be determined, renders the information obtained by it incomplete. The isolated heart has at its disposal, for the production of energy, in addition to the dextrose offered to it in the perfusion fluid, its own store of reserve materials, of which glycogen is the most important. When we are dealing with a complex in which, on the one hand, dextrose disappearing from the perfused fluid may be either burnt or otherwise changed, while the CO_2 collected may be due to the combustion of either circulating dextrose or previously stored material, it is obvious that the figures obtained for disappearance of dextrose and output of CO_2 represent only the balance of the different processes. When, however, the CO_2 produced is more than what the combustion of the disappearing dextrose would afford, it is reasonable to suppose that the lost dextrose is all burnt, and stored material in addition, and this is the relation usually, though not invariably, found, when the heart, whether normal or from a diabetic animal, is perfused without addition of insulin. When, on the other hand, more dextrose disappears than the CO_2 produced will account for, it is certain that all the lost dextrose has not been burnt, but that some has been changed into some other form, and this is the relation commonly found when insulin is added to the perfusion fluid. We can state with certainty, therefore, that some of the additional dextrose which disappears under insulin is not oxidised, the point which is not settled beyond question is whether any of it is. In these respects, our experiments on hearts from animals rendered completely diabetic by pancreatectomy give results quite similar to those obtained with hearts from normal animals.

PART II. EXPERIMENTS ON THE DECAPITATED AND EVISCERATED CAT

Methods Decapitation was carried out under ether by Sherrington's method, and from the spinal preparation the abdominal portion of the alimentary canal was then removed, ligatures being applied successively to both mesenteric and the coeliac axis arteries, the lower ends of the rectum and œsophagus, and finally the portal vein. The kidneys were then tied off and removed. It is not practicable to extirpate the liver in the cat, but, the afferent vessels being tied and cut, its vascular system formed a mere "cul de sac" on the vena cava. That no effective interchange occurred, between the blood there stagnating and that in circula-

tion, was sufficiently evident from the rapid and steady fall of blood-sugar which occurred in this preparation, as compared with the long-maintained hyperglycæmia of the simply decapitated animal. Essentially it could be regarded as a preparation consisting of heart, lungs, skeletal muscles and skin. Since the blood-sugar falls steadily and rapidly in such a preparation, it was necessary, in order to study the effect of insulin, to maintain its level artificially. This was effected by very slow, steady infusion into a jugular vein of solutions containing from 1 to 4 p.c. of dextrose in physiological saline solution. The device used to secure a constant, very slow flow from the burette may be mentioned, as it may be useful for other purposes. The burette being filled to the mark with the dextrose solution, the ungraduated upper portion was filled with a thick lubricating oil, and closed by a rubber cork, through which passed a thick-walled glass tube, with capillary bore (thermometer tubing), connected by a length of thick-walled rubber tubing to a glass reservoir, filled with the same oil. The rubber connecting tube and the capillary being filled with the oil before insertion of the cork, and air-bubbles completely excluded, the rate at which the solution can issue from the lower end of the burette is entirely governed by the rate at which the viscid oil can enter the upper end through the capillary (see Fig. 1). The latter rate is fixed within a certain wide range by choice of a capillary tube of suitable bore and length, and within this range adjustment can be made by raising or lowering the reservoir, or by creating a positive pressure in it, for which purpose it is furnished with a hand-bellows and mercury gauge. When the air space of the reservoir is open, the flow is governed by the hydrostatic pressure, which remains practically unchanged as the infusion proceeds, being diminished only by the trivial fall of the oil level in the reservoir, and the replacement of the sugar solution by the somewhat lighter oil in the burette. This can be sufficiently compensated by very slightly raising the reservoir during

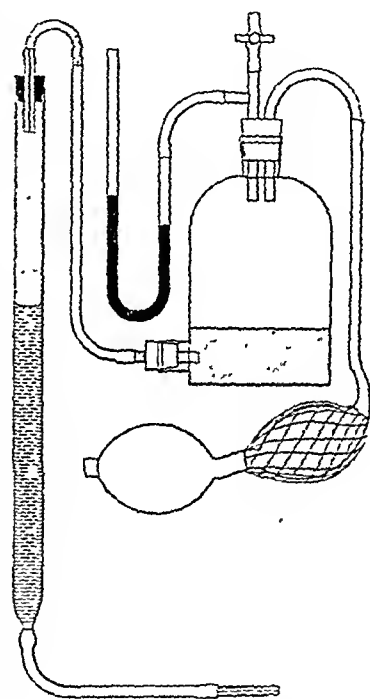


Fig. 1. Slow infusion apparatus.
For description see text.

the progress of the infusion; but the flow, even without such adjustment, can be kept constant at such a rate as 1 c.c. in 10 minutes, with a variation between successive periods of not more than ± 0.05 c.c.—i.e. the accuracy is that of the burette reading. The rate can be increased to three, four or five times this by creating the appropriate positive air-pressure in the reservoir, and will again remain steady at the higher level, so long as the air-pressure is kept constant. On the other hand, by lowering the reservoir, with its air-space open, the rate of infusion can be further reduced and yet remain perfectly steady. The one point needing care is to see that no clot in the cannula or kinking of the vein is allowed to create a resistance greater than that presented by the capillary tube to the passage of the viscid oil.

For experiments on the respiratory metabolism we used the ingenious circulating respirometer made by our colleague, Dr E. J. Schuster, who originally designed it in connection with an investigation projected in this Department by Prof. Lovatt-Evans. The only modification made for our purpose was to discard the soda-lime container fixed to the wedge-shaped gasometer, and to insert in the system a separate tower of soda-lime, and alternatively, in experiments in which CO_2 output was determined as well as O_2 consumption, a train of absorption-tubes, in which the weighed soda-lime tubes were preceded by a large vessel containing sulphuric-acid pumice, and followed by weighed tubes of the same. In complete metabolism experiments a triple absorption system was introduced, the circulating air being passed through the simple soda-lime tower till the oxygen-absorption recorded by the counter had become steady, switched for a control period through the first train of weighed tubes, and then, immediately after injection of insulin, into a second similar train for the insulin period.

In all experiments the preparation was fixed upon an electrically heated table, thin sheets of cork being inserted between the warm plate and the hair of the back, and the ventral surface and sides covered with cotton wool and cloths. A thermometer bulb was inserted, through a small skin incision on the chest, into the loose tissue of the axilla. During the control periods, without insulin, the temperature remained reasonably constant, varying only a few tenths of a degree. In some experiments, when the administration of insulin was followed by a definite acceleration of metabolism, the temperature began to rise quickly, and it was necessary to turn off the heating-lamps for the time being. Such an adjustment was rather crude, but it appeared to be the best available, since immersion of the preparation in a warm saline bath, with the long abdominal wound

giving access to the empty peritoneal cavity, would have afforded indefinite opportunity for the diffusion of dextrose from the tissues into the bath. The arterial pressure was recorded from one carotid artery, with a mercury manometer, the other being used for the taking of blood samples, 1 c.c. being taken at each bleeding. Insulin was injected, in a volume of 1 c.c. or less, into the internal saphena vein by means of a hypodermic needle. Two samples of insulin hydrochloride were used, one containing a rabbit-convulsant dose in about 0.4 mgm., the other in about 0.25 mgm. The former produced a quite notable rise of the arterial blood-pressure, lasting for about 15 minutes, which we were inclined for a time to regard as a true, physiological concomitant of the insulin action on the decerebrated preparation. The fact that the second, more powerfully insulin-active sample had a relatively negligible pressor effect, showed that this action must be due to some other constituent, and had no true connection with insulin.

Location of the effect. Our first object was definitely to locate the organs responsible for the disappearance of dextrose from the blood under insulin. Olmsted and Logan had shown that the effect was normally produced in the decapitated preparation, and this we confirmed. Preparations in which the stomach, intestines and kidneys were removed, and the liver excluded from circulation, were then made, and showed a decided acceleration of the decline in the blood-sugar percentage, following the injection of insulin. The normal decline, however, under such conditions was usually rapid. We accordingly made other experiments in which, by constant slow infusion of dextrose, the blood-sugar was brought to and held at a practically steady level. Insulin being then injected, with the infusion of dextrose unchanged, an abrupt fall in the blood-sugar took place, until, after one to two hours, a new level was reached, at which the blood-sugar appeared again to become approximately constant. A few records will illustrate the course of events.

Exp. 1. (See Fig. 2.) Cat, 2.6 kgm. Decapitated under ether anæsthesia. Thyroids excised. Eviscerated. Kidneys and suprarenals also removed. Continuous infusion of 4 p.c. dextrose into the l. ext. jugular vein. At 2 p.m., 10 physiological units of insulin were injected by intravenous route.

Time	1.15	1.35½	1.57½		2.26	2.46	3.3	3.33
B. sugar in p.c.	0.176	0.166	0.156	↓	0.068	0.05	0.04	0.04

Exp. 2. Cat, 2.4 kgm. Prepared as in Exp. 1. 10 physiological units of insulin injected at 1.15 p.m.

Time	12.18½	12.49	1.14½		1.35½	1.56½	2.28
B. sugar in p.c.	0.208	0.204	0.209	↓	0.188	0.124	0.110

The curve obtained is similar to that observed in a well-fed animal receiving a large dose of insulin. Under the conditions of these experi-

ments, however, we obtain data for a fairly accurate calculation of the change effected by insulin, in the rate at which sugar is removed from the blood by the tissues. To make such an estimate, we need a figure for deducing the total amount of circulating sugar, from the content of a blood-sample. We must take into reckoning not merely the volume of the blood itself, but that of the fluid in the lymph and tissue-spaces, which presumably keeps in approximate sugar-equilibrium with the blood-plasma. The fairest method of arriving at such a figure seemed to be that of an experiment, which we carried out. A cat weighing 3.2 kgm.

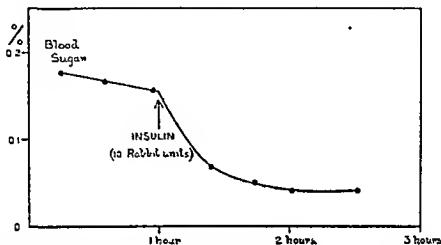


Fig. 2. Cat, decapitated and eviscerated. Constant infusion of dextrose. Effect of insulin on blood sugar.

was decapitated and eviscerated in the usual way, and a 4 p.c. solution of dextrose was infused until the blood-sugar became steady at 0.286 p.c. The rate of infusion maintaining it at that level was 1.5 c.c. of the 4 p.c. dextrose in 10 minutes. After half an hour at this rate the infusion was suddenly accelerated, so that in the next 15 minutes 8.25 c.c. were infused. This acceleration caused a rise of blood-sugar from 0.288 to 0.334 p.c. From the previous 30 minutes, we know that 2.25 c.c. would be needed to keep the percentage steady for 15 minutes, provided the rate of sugar usage remained constant. During such a short period we have no reason to believe that it would change to such an extent as to affect the calculation materially. The quantity used in raising the percentage from 0.288 to 0.334 is, therefore, $8.25 - 2.25 = 6$ c.c. of 4 p.c. dextrose, or 240 mgm. of dextrose. This causes a rise of only $334 - 286 = 48$ mgm. per 100 c.c. So that the volume into which it is delivered and distributed may be reckoned as $\frac{240 \times 100}{48} = 500$ c.c. This is the volume which we have adopted for our calculations, in dealing with cats of approximately 3 kilos., with a proportionate reduction in the case of smaller ones. Thus, if the blood-sugar of a 3 kilo. cat falls by 50 mgm. p.c.

when none is being added by infusion, we reckon that the free, circulating sugar, which has disappeared from the system is $\frac{50 \times 500}{100} = 250$ mgms. Applying this calculation to Exp. 1, on a cat weighing 2.6 kgm., we take the effective volume as 433 c.c., and obtain the following figures for rates of sugar removal.

Before insulin. Blood-sugar falls 20 mgm. p.c. in $42\frac{1}{2}$ mins., during infusion of 5.1 c.c. of 4 p.c. dextrose.

$$20 \times 433 \div 100 = 87 \text{ mgm.}$$

$$5.1 \times 40 = 204$$

$$\text{Total } 291 \text{ mgm. in } 42\frac{1}{2} \text{ mins.} = 410 \text{ mgm. per hour.}$$

After insulin. Blood-sugar falls 106 mgm. p.c. in $45\frac{1}{2}$ mins., during infusion of 5.88 c.c. of 4 p.c. dextrose.

$$106 \times 433 \div 100 = 459 \text{ mgm.}$$

$$5.88 \times 40 = 235$$

$$\text{Total } 694 \text{ mgm. in } 45\frac{1}{2} \text{ mins.} = 914 \text{ mgm. per hour.}$$

A much more striking increase in the rate of sugar disappearance, as the result of injecting insulin, could be produced if, instead of maintaining the rate of infusion constant, it was accelerated shortly after the insulin had been injected, so as to prevent the fall, or restrict it to relatively small limits.

The results of one such experiment (Exp. 3) are shown diagrammatically in Fig. 3. In the preliminary period, the cat, after preparation

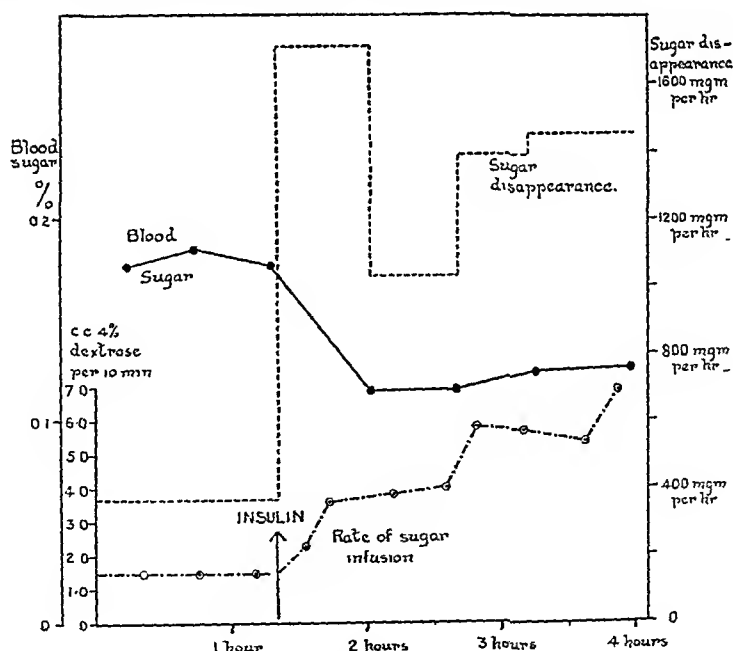


Fig. 3.

as was described in Exp. 1, received an infusion of a 4 p.c. dextrose at a steady rate of 1.55 c.c. in 10 minutes. Insulin (six rabbit convulsant doses) was injected, and the infusion rate was raised to over three times the previous value, but in spite of this the blood-sugar was observed to fall.

Applying the same method of calculation as before, we obtain for the 40 minutes of the pre-insulin period, and the four successive periods of 20 minutes each, after insulin, the following rates of sugar-disappearance in mgms. per hour.

Before insulin	369
1st 20 mins. after	1704
2nd " "	1026
3rd " "	1392
4th " "	1452

It was clear, therefore, that when the sugar-percentage in the blood is prevented from falling below the normal level, the acceleration of sugar-removal by insulin is relatively enormous. It became of interest to study the respiratory metabolism under such conditions. But, before proceeding to these experiments, some other points in the more definite localisation of the effect may be mentioned. It will be noticed that in Exp. 1 the thyroid gland and the suprarenals were removed; in another case the gonads also were excised. This removal of the endocrine glands made no difference to the effect of insulin. Finally we may describe two further experiments, on the nature and function of the tissues concerned in the removal.

Exp. 4 was made on a preparation which, after decapitation and evisceration in the usual way, was completely skinned, each portion of the body being abundantly smeared with vaseline, and wrapped in layers of cotton wool, as the skin was removed. The swathed carcass, kept on the warm table, maintained its temperature quite adequately during the two hours of the experiment, the thermometer in the abdomen registering 36.1° C. at the end. Four p.c. dextrose was infused at the rate of 1.5 c.c. per 10 minutes, and maintained the blood-sugar steady at 0.256 p.c. for an hour before insulin was injected. Twenty rabbit units were then injected, and, with the same rate of infusion, the blood-sugar fell to 0.112 p.c. in an hour.

Exp. 5 was made to exclude the effect of the tone and slight, irregular activity shown by the muscles even in the spinal preparation. The preparation was decapitated and eviscerated and the infusion of 4 p.c. dextrose begun and maintained throughout at 1.2 c.c. per 10 minutes.

Two c.c. of a 1 p.c. curare extract were then injected intravenously. The efficiency was tested, and confirmed late in the experiment, by faradisation of the sciatic nerve, without response from the muscles, which responded immediately to direct faradisation. The blood-sugar in the 45 minutes before insulin rose from 0.252 to 0.276 p.c. Twenty rabbit-units were then injected and, with maintained infusion, the blood-sugar fell in 1 hour to 0.098 p.c., and in a further 30 minutes, to 0.075 p.c.

These experiments have made it clear that, in the absence from circulation of all viscera, except the heart and lungs, the body wall and limbs are able to cause the disappearance of dextrose from the circulation under insulin in a perfectly normal manner; that the same occurs when the skin is further removed, leaving a preparation consisting of heart, lungs, bones and skeletal musculature; and that the complete exclusion of muscular tone and activity by curare, produces, at any rate, no qualitative change in this action of insulin. The amount of sugar disappearing is so large that the heart could not account more than a negligible portion of it, and it is not likely that the lungs and the bones could make a significant contribution to the effect. The conclusions seem warranted that the skeletal muscles are largely responsible for the disappearance of dextrose under insulin, and that they can perform this function when completely at rest.

Effects on respiratory metabolism. The use of the decapitated and eviscerated preparation, with intravenous infusion of dextrose, gave us the opportunity of examining the changes in respiratory metabolism, accompanying an acceleration in the removal of dextrose from the blood which could be estimated with reasonable accuracy.

Experiment without insulin. There was one point which it was desirable to settle first, namely, the nature and extent of the changes produced in the respiratory metabolism, of such a preparation, by changes in the concentration of the blood-sugar, produced independently of insulin. Several experiments were carried out in which the level of the blood-sugar was either raised, by accelerating the infusion, or lowered by stopping the infusion, and allowing the normal consumption progressively to deplete the blood.

Exp. 6. Cat, 3.1 kgm. Decapitated, eviscerated and kidneys excised. Infusion of 4 p.c. dextrose into l. ext. jugular vein, during a preliminary period of 50 mins. At the beginning, in the middle and at the end of this time, the blood-sugar values were 0.414, 0.420, 0.420 p.c. respectively. The oxygen consumption for each 5 mins. of the same period showed only the small variation from 62 c.c. to 58 c.c. The infusion of dextrose was then stopped. The blood-sugar fell in 23 mins. to 0.304, and in the same period the oxygen consumption fell to 54 c.c. per 5 mins. During a further period of 75 mins. the blood-sugar

fell to 0.240 p.c. and the oxygen consumption to 40 c.c. per 5 mins. This fall in oxygen consumption was not secondary to a fall in temperature. By increase of the heat supplied by the table the axillary temperature was actually raised in the course of the experiment from 38.6° C. to 39.2° C.

It will be seen that a fall of the sugar percentage in the blood, produced by simple stoppage of the supply, is accompanied by a definite diminution in the rate of oxygen absorption, even when the sugar percentage remains throughout the period of observation above the level seen in the normal animal. Conversely, a rise in the percentage, from a level above the normal to a higher one, is associated with an increase of oxygen absorption. Accordingly we cannot, in an experiment with insulin, attribute much significance to an increase in absorption of oxygen, if the infusion is so accelerated that the percentage of sugar in the blood is made to rise, in spite of insulin. On the other hand, when the level of blood sugar is allowed to fall, as it will do after insulin when the infusion is kept constant, even a failure of the oxygen absorption to show a concurrent fall will have a significance of its own.

Experiments with insulin. In some of these only the rate of oxygen usage was recorded, absorption of CO₂ being carried out by a plain, unweighed tower of soda-lime. The following are examples.

Exp 7 Cat, 2.8 kgm. Prepared as in previous experiments. Infusion of 2 p.c. dextrose. Before injection of insulin in the preliminary period of 40 mins. the oxygen consumption was regular, the figures for c.c. in successive 5 min. periods being 44, 44, 48, 48, 48, 48, 48. The temperature fell slightly from 36.4° C. to 36.1° C. The blood sugar fell slightly from 0.262 p.c. to 0.227 p.c. The blood pressure fell from 100 mm. to 94 mm.

After injection of 7 convulsant rabbit doses the oxygen consumption rose slightly as shown by the sequence 46, 52, 52, 52, 52, 52, 52, 52, 54, 52, 50, 48 (65 mins. after insulin). The temp. rose slightly from 36.1° C. to 36.9° C. The blood sugar fell in 25 mins. from 0.227 p.c. to 0.170 p.c. and in 54 mins. to 0.128 p.c. The blood pressure, apart from a rise from 94 mm. to 100 mm., immediately following the injection and lasting less than 1 min., fell from 94 mm. to 64 mm. in the 24 mins. following the injection, and to 52 mm. in a further period of 36 mins.

Exp 8 Cat, 3.9 kgm. Preparation as in previous experiments. Infusion of 1 p.c. dextrose. The results are shown in Fig. 4. The increase of oxygen consumption following insulin, from about 60 c.c. per 5 mins. up to a maximum of 106 c.c. per 5 mins., is the largest recorded in any of our experiments. The insulin used in this experiment was a pressor sample, causing a rise of blood pressure, not shown in Fig. 4, from 56 mm. to 132 mm. of mercury in 3½ mins., returning to the original level in 24 mins. In Exp. 7, no such rise took place, but a fairly rapid fall. It is certain that the unusually large rise of oxygen consumption seen in Exp. 8, is not due to the non-specific pressor action. Other experiments with the same pressor sample of insulin showed as extensive rises of arterial pressure, with only moderate increases of oxygen consumption, such as that seen in Exp. 7 with a depressor effect. The rise in oxygen intake, in any case, long outlasts the pressor effect, and no similar increase in the oxygen absorbed is seen when other pressor substances, producing larger and more lasting rises of arterial pressure, are injected.

The fall in the percentage of blood-sugar following the injection of insulin has in no case been accompanied by a definite fall in the consumption of oxygen, such as occurs when the blood-sugar falls from failure

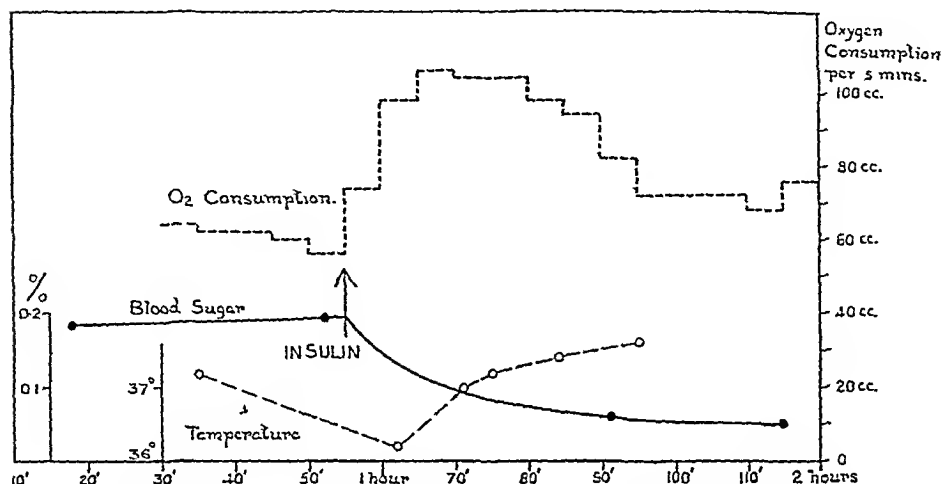


Fig. 4. Unusually large increase of O₂ consumption following insulin.

of supply. In some cases it was practically unchanged, until the blood-sugar had become really low, when it showed a slight fall. In most cases there was a definite increase in the consumption of oxygen during the first period of the action of insulin, when the blood-sugar is falling most rapidly, and in a few cases the increase is pronounced. Exp. 8 (Fig. 4) shows the most striking example of an increase in oxygen consumption, produced by insulin, in our records; but there are many in which the effective rise is significant, in view of the fact that a similar fall of blood-sugar, without insulin, would be attended by a pronounced fall in the oxidative metabolism.

We may conclude, therefore, that the removal of sugar from circulation by insulin under the conditions of these experiments involves an oxidative factor. There remains, however, the question whether the observed increase in oxygen consumption is sufficient to account for the removal by combustion of all the extra sugar which disappears. An examination of the figures gives a definitely negative answer. We may choose examples.

Exp. 9. Cat, 2.05 kgm. Before injection of insulin. In spite of the continuous infusion of dextrose the blood-sugar fell from 0.200 p.c. to 0.176 p.c., i.e. by 0.024 p.c. in one hour. The calculation volume for this cat would be 342 c.c. (see p. 179). So that there was a disappearance of sugar of $\frac{0.024}{100} \times 24 \text{ mgm.} (= 82 \text{ mgm.})$ per hour in addition to the sugar infused.

In one hour 6 c c of 2 p c dextrose were infused = 132 mgm The total sugar disappearance is $132 + 82 = 214$ mgm per hour

During the half hour previous to the injection of insulin the oxygen intake was at the rate of 315 c c per hour, while the oxygen needed to burn the sugar which disappeared would only have been 160 c c per hour

After the injection of insulin The rate of infusion was maintained, but the percentage of sugar in the blood fell from 0.168 to 0.067 in 44 mins $\frac{7\frac{1}{2}}{14} \times \frac{1\frac{1}{2}}{1\frac{1}{2}} \times 101 = 471$ mgm per hour The rate of infusion was still 132 mgm per hour Hence the total sugar disappearance was 603 mgm per hour

During the half hour following the injection of insulin the oxygen usage was at the rate of 386 c c per hour The oxygen needed to burn the sugar which disappeared would have been 450 c c per hour The results for this experiment, and for another similarly calculated, may be tabulated

TABLE VII Exps 9 and 10

	Before insulin			After insulin		
	O ₂ (obs)	Dextrose	O ₂ (calc)	O ₂ (obs)	Dextrose	O ₂ (calc)
Exp 9	315	214	160	386	603	450
Exp 10	364	293	218	413	1736	1295

The figures represent mgms of dextrose or c c of O₂ per hour O₂ (calc) = amount of O₂ required to burn the dextrose which disappeared

It is clear that the excess consumption of oxygen, during the maximum period of sugar removal, is not usually sufficient for the combustion of more than a small part of the extra sugar which disappears, and that, therefore, a large part of the sugar must be dealt with in some other way. In Exp No 8, on the other hand, quoted above and illustrated by Fig 4, the exceptionally large increase in oxygen intake during the 40 minutes after insulin seems to be compatible with complete oxidation of the sugar which disappears. We have not complete data for the rate of sugar disappearance, in this experiment, before insulin was injected, but for the first 40 minutes of the insulin period, calculation by the usual method gives us the following

$$\begin{aligned}
 \text{Dextrose infused} &= 4.8 \text{ c c of } 2 \text{ p c} &= 96 \text{ mgm} \\
 \text{Blood sugar fall} &= 0.130 \text{ p c on a volume of } 650 \text{ c c} &= \frac{845}{100} \\
 \text{Total dextrose disappearing} &= 941 \text{ mgm} \\
 \text{O}_2 \text{ consumption observed during same period} &= 760 \text{ c c} \\
 \text{O}_2 \text{ needed for combustion of the dextrose} &= 703 \text{ c c}
 \end{aligned}$$

The assumption on which the calculated sugar disappearance is based is so crude that no significance attaches to such a small discrepancy between the calculated and observed oxygen figures. If we assumed a volume of 700 c c, instead of 650 c c, the calculated and observed values for oxygen would be practically identical. This result is accordingly not incompatible with the view that all the sugar which disappears is immediately

oxidised, if we further assume that the whole of the oxygen consumed is used for this purpose. But, even with such an assumption, this is the only example among our records in which the figures would bear this interpretation. Even in this experiment, the *additional* O₂ consumed in the 40 minutes following the injection of insulin, as compared with those immediately preceding it, viz. 278 c.c., is less than one half of what would be needed for combustion of the *additional* sugar lost.

Among the other possibilities for the non-oxidative removal of sugar is its conversion into fat. This would be very difficult to detect by direct analytical methods, if it were spread, as it presumably would be, over the whole of the muscles of the trunk and limbs. It ought, however, to be made obvious by the respiratory exchange, since the immediate conversion into fat, of the quantity of sugar not represented by the oxygen consumption, would involve a definite rise in the respiratory quotient. We accordingly carried out experiments in which the CO₂ produced was collected and weighed. After a preliminary period in which only the soda-lime tower was used, until uniformity of infusion and of oxygen absorption had been assured, the respiratory circulation was switched into a train of weighed absorption tubes for a period of 20–40 minutes, and a blood sample was taken for sugar estimation. One minute before the end of this period a second blood-sample was taken, and the dose of insulin was injected. The circulating air was then switched to the second train of weighed tubes for a second similar period. Table VIII gives results of experiments carried out in this way.

TABLE VIII.

	Before insulin					After insulin				
	CO ₂	O ₂ (obs.)	R.Q.	O ₂ (calc.)	Dextrose	CO ₂	O ₂ (obs.)	R.Q.	O ₂ (calc.)	Dextrose
Exp. 11	476	465	1.02	262	351	447	489	0.92	585	783
Exp. 12	630	594	1.06	—	—	615	622	0.99	744	996
Exp. 13	437	441	0.99	332	444	504	498	1.01	762	1021

CO₂ and O₂ in c.c. per hour. O₂ (calc.) = No. of c.c. needed for complete combustion of dextrose. Dextrose in mgm. per hour.

It will be seen that, in every case the respiratory quotient, before the injection of insulin, was almost unity. It is quite possible that such a quotient may be characteristic of muscle, when isolated, as in our experiments, from other vigorously metabolic tissues, and that it may indicate the use by muscle of dextrose alone as the source of its energy requirements, other food-stuffs, fat, protein, being so used by muscle only in so far as dextrose is previously formed from them in the liver.

On the other hand, the respiratory quotient of 1 might be attributed to the fact that we were always offering to the muscle dextrose in excess. However that may be, with a normal quotient in the neighbourhood of unity, the superimposition of a process involving the immediate conversion of large quantities of dextrose into fat would necessarily raise the quotient well above unity. No such change is observable in any of our records; the oxygen intake is in every case somewhat increased, but the CO_2 production shows a slight fall in Exps. 10 and 11, and in Exp. 13 shows an increase only slightly greater than that of the oxygen intake. As a result, the respiratory quotients show no certainly significant changes. Whatever becomes of the sugar which is not oxidised, therefore, we can regard the possibility of its immediate conversion into fat as excluded. These results would, further, be difficult to reconcile with the suggestion of Briggs, Koechig, Doisy and Weber, that the immediate fate of the sugar disappearing under insulin is conversion into lactic acid. In Exp. 13, the only one of the above experiments in which the CO_2 output shows any increase, the sugar loss is increased by 577 mgm. per hour. If this extra sugar were converted into lactic acid, it should liberate, from the carbonates, about 144 c.c. of CO_2 without any corresponding oxygen intake. The actual increase of CO_2 recorded is 67 c.c., of which 57 c.c. correspond to increased oxygen intake. The other two experiments show actually a slightly lower figure for CO_2 than for oxygen after insulin.

Experiments on diabetic animals. As in the case of the experiments on the isolated heart, a few cats were rendered diabetic by complete removal of the pancreas, and two days later, when the animal already showed evidence of advanced diabetes, the decapitated eviscerated preparation was made. If the cats were kept for a longer period, until somnolence appeared, we found it impossible to obtain such a preparation with an efficient circulation; but the condition following the pancreatectomy was so rapidly progressive, that we felt it safe to assume that, after two days, when successful preparations could be made, the animal was already insulin-free.

The initial blood-sugar of these preparations was over 0.3 p.c., but to keep it at a high level it was necessary to give a slow constant infusion of 1 p.c. dextrose. The respiratory metabolism was recorded in the control period and after insulin, as with the preparations from normal cats. We quote two experiments in one of which only the oxygen consumption is recorded, while in the other the CO_2 output was also determined, so that the respiratory quotients could be calculated.

Exp. 14. Mar. 20th. Cat, 2 kgm. Pancreatectomy under ether anaesthesia with full aseptic precautions. Mar. 21st. Cat well. 2.5 p.c. sugar in urinc. Mar. 22nd. 5.2 p.c. sugar in urine.

Ether. Tracheotomy; carotid arteries tied; decapitated and given artificial respiration; eviscerated and kidneys removed. Infusion of 2 p.c. dextrose into l. ext. jug. vein; l. saph. vein prepared for injection. Blood-pressure record taken from the right carotid, and samples of blood for sugar determinations from the l. carotid.

Before injection of insulin. The blood-sugar remained 0.322 p.c.; the temperature 35.5° C.; the blood-pressure 60 mm. The oxygen consumption during 30 mins. was 177.3 c.c., CO₂ production 186.9 c.c., and the respiratory quotient was 1.05.

After injection of insulin. The blood-sugar fell in 40 mins. to 0.180 p.c.; the temperature fell slightly to 35.2° C.; the blood-pressure rose to 150 mm. returning to 70 mm. in 25 mins. and continued to fall. The oxygen consumption during 40 mins. was 185.2 c.c., CO₂ production 187.3 c.c., and the respiratory quotient was 1.01.

Exp. 15. Feb. 18th. Cat, 2.5 kgm. Pancreatectomy. Urine contained 2.5 gm. dextrose on the 19th, and 5.4 gm. on the 20th. Prepared as described in last experiment.

Before injection of insulin. Temperature constant at 38.1° C. Blood-pressure fell from 58 mm. to 54 mm. Blood-sugar fell in 55 mins. from 0.354 p.c. to 0.283 p.c. Oxygen consumption during 55 mins. amounted to 519 c.c.

After injection of 25 convulsant rabbit doses. Temperature rose to 38.5° C. Blood-pressure rose to 102 mm., but fell in 25 mins. to 56 mm. once more. Blood-sugar fell from 0.283 p.c. to 0.086 p.c. in 90 mins. Oxygen consumption during the first 55 mins. rose to 559 c.c.

The most striking thing about these records is their similarity to those obtained with preparations from normal cats. The rate of metabolism in the control period is less than what would be expected, at such high blood-sugar level, from the skeletal muscles of normal animals; but the metabolism is quite normal in type, the respiratory quotient being unity. After insulin there is again a small increase in the metabolic rate, instead of the decline which would accompany a similar fall of blood-sugar produced by stoppage of the supply, and even when the blood-sugar has sunk to well below the normal level, the metabolism is identical with that which before insulin accompanied a much higher proportion of sugar in the blood. In this respect the results are closely parallel to those obtained in the preparations from normal cats.

DISCUSSION.

The action of insulin, on either the normal or the diabetic organism, is probably a complex of a number of factors. The deduction of the contributory factors from the crude resultant effect observed in the whole animal, is a speculative procedure. What we have here attempted is the artificial isolation of one part of the mechanism, with the object of investigating the nature of the factors concerned in the immediate disappearance of sugar from circulation, under the influence of an

excessive dose of insulin. The process revealed by such a form of experiment can reasonably be assumed to represent an important stage in the effect on the whole animal; but it cannot be assumed to be more than that, and its precise significance, in relation to processes in organs here deliberately excluded, must be left for future investigation. Within these limits we can draw conclusions, which will by no means provide a complete theory of insulin action, but will suffice to exclude certain general theories which have been put forward on evidence of other kinds.

Our experiments show that the initial stage in the metabolic sequence initiated by insulin, the disappearance of dextrose from circulation, can occur quite normally in the absence of all tissues with an important metabolism, except the heart and the skeletal muscles. This does not exclude the possibility that other organs, such as the liver, may be equally concerned in this process, as seen in the whole animal; but it does not allow us to regard any of them as playing a special and indispensable part in it. For example, it makes it impossible to suppose that an essential step in the disappearance of dextrose is its conversion by the liver, under the influence of insulin, into a reactive form. Ordinary dextrose, infused straight into the heart-lung-muscle preparation, disappears under insulin like the normal blood-sugar.

This observation appears to give a definite negative, on the other hand, to such an hypothesis as that of Laufberger, which supposes that insulin simply stops the new formation of sugar from fat, while the sugar already present as such continues to be used at the normal rate. Our experiments show that the removal from circulation of artificially infused dextrose may be accelerated by insulin to a rate many times the normal; the fall of blood-sugar is certainly due, therefore, to acceleration of removal, and not to restriction of supply.

As to the fate of the dextrose which thus disappears our information is still very incomplete, and, in default of adequate evidence as to their meaning, we do not propose at this stage to discuss the suggestive observations of several workers on the concurrent changes in blood phosphate, etc. From our own experiments we feel entitled to conclude that, when the supply of sugar is plentiful and maintained, the first stage of insulin action is accompanied by accelerated oxidation which, however, is not sufficient to account for more than a part of the sugar which disappears. About a year ago one of us put forward a suggestion, to explain the effects of excess of insulin on the metabolism of the intact animal, according to which the initial rise of the respiratory quotient, accompanied by increase in total oxidation, might be attributed to a

concentration of metabolism on the carbohydrate, and the sparing of an equivalent of protein and fat. This suggestion is quite in conformity with the observations on the respiratory metabolism of the curarised rabbit under insulin made by Krogh, and on that of human patients by Kellaway and Hughes, and by Lyman, Nicholls and McCann. The suggestion is so far in harmony with the results here under consideration, that in the case of the musculature metabolising dextrose alone, insulin causes a definite, though small and variable increase in the respiratory metabolism, without significant alteration of the quotient. To account for the disappearance under insulin of much more sugar than the oxidation accounts for, some further process is required. Until the form into which this sugar is converted has been directly identified, no positive conclusion is warranted; but it is clear that our results could be produced by a coupled reaction such as that postulated for the whole animal by Bissinger, Lesser and Zipf, in which, when the sugar supply is large in relation to the insulin dosage, a part of the sugar is burnt, and the energy so liberated used in the synthesis of a further quantity into glycogen. M. Ringer has also shown that insulin will cause both combustion of dextrose and its storage, presumably as glycogen, in dogs so completely under the influence of phloridzin as to be unable otherwise to burn or to store any. The experimental search for evidence of such a process in the eviscerated preparation is the next step required. Our determinations of the respiratory quotient appear to exclude the immediate transformation of the sugar into fat or lactic acid. With regard to our results on preparations made from diabetic animals, our results agree with those of Lesser, Parnas, Macleod and Pearce, in showing that removal of the pancreas does not affect the power of the resting muscle to use dextrose, and that the effect of the muscles of depancreatized animals, whether cardiac or skeletal, on the blood-sugar is changed by insulin in a manner which shows no qualitative or quantitative difference from that observed with the muscles of normal animals. The obvious conclusion seems to be that there is no significant store of insulin in the muscle of the normal animal, and that the distinction between normal and diabetic ceases with removal from the conditions of normal interchange with the viscera, whether by isolation from the body, in the case of the heart, or by evisceration of the animal, in the case of the skeletal muscles.

SUMMARY AND CONCLUSIONS.

The following are the main results of the investigations:

1. Addition of insulin to the perfusion fluid accelerates the removal of dextrose therefrom by the isolated mammalian heart (confirming Hepburn and Latchford).

2. The output of CO_2 by the heart is not proportionately increased, so that the extra loss of sugar is not entirely due to increased oxidation.

3. In the decapitated and eviscerated cat, with constant infusion of dextrose, insulin produces the characteristic fall of blood-sugar.

4. If the blood-sugar is prevented from falling by accelerating the infusion, insulin causes disappearance of sugar at many times the normal rate.

5. The earlier stages of the insulin action are accompanied by increased consumption of oxygen, which may be pronounced, but is seldom, if ever, sufficient for the oxidation of the extra sugar lost.

6. The respiratory quotient of such a preparation has always been unity, and has remained practically unchanged under the action of insulin. Immediate conversion of the sugar into fat or lactic acid seems to be excluded.

7. The isolated hearts and eviscerated preparations, from cats rendered diabetic by complete pancreatectomy, have given results in all respects similar to those from normal animals.

8. The significance of these observations in relation to current theories is discussed.

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THE EFFECT OF INSULIN ON BLOOD VOLUME.

By J. B. S. HALDANE, H. D. KAY (*Beit Memorial Research Fellow*) AND W. SMITH (*Beit Memorial Research Fellow*).

(*From the Biochemical Laboratory, Cambridge.*)

DURING the course of an investigation into the effect of insulin on the organic phosphorus compounds of blood and muscle, Kay and Robinson⁽¹⁾ found, using normal rabbits as the experimental animals, that there was an increase in the "lactacidogen" phosphorus in muscle after insulin greater than could be accounted for by the diminution in inorganic phosphate in the blood reported by Wigglesworth, Woodrow, Winter and Smith⁽²⁾ and by other workers^(3, 4). The total acid-soluble phosphorus in 100 c.c. blood was found to be less after insulin, but the diminution was comparable with the extent to which the inorganic phosphorus (which is comprised in the total acid-soluble phosphorus) was decreased. This point is still under investigation by Kay and Robinson, but it is beyond doubt that there is no very great change in the organic portion of the total acid-soluble phosphorus after insulin. Some other source had therefore to be found for the increase in phosphorus in muscle which the rise in lactacidogen phosphorus appeared to indicate. Although this increase in lactacidogen phosphorus has since been found to take place at the expense of other phosphorus-containing compounds present in muscle, and therefore not of necessity immediately associated with a diminution in any of the phosphorus compounds in the blood, it led the present authors to investigate changes in the "lecithin phosphorus" of blood, which, according to the careful experiments of Bloor⁽⁵⁾, is the only form of phosphorus present in blood in significant amounts that is not present in the acid-soluble fraction. Estimations of the lecithin phosphorus on 2 c.c. of blood taken from the ear vein of rabbits before and after the administration of insulin were made, using a modification of the method of Randles and Knudson⁽⁶⁾ which is itself founded on Bloor's original method. At first it seemed clear that there was a definite decrease in the amount of lecithin in the blood after insulin.

Lecithin phosphorus in mgm. per 100 c.c. whole blood			Remarks
Control	After insulin	Decrease p.c.	
9.4	8.8	+ 6	Convulsions after 5 hrs.
12.4	9.4	+ 25	Convulsions after 3 hrs.
10.6	11.8	- 11	{ Effect of insulin very rapid. Convulsed after 1 hr.
10.2	7.6	+ 25	After 2 hrs. no convulsions
11.6	7.6	+ 34	After 2½ hrs. no convulsions
11.6	9.2	+ 21	After 5 hrs. no convulsions
10.0	9.4	+ 6	Convulsions after 1½ hrs.
Av. 10.8	9.1	16	

"After insulin" blood sample taken after the period of time mentioned in the remarks column.

There was thus, on the whole, a 16 p.c. decrease in the amount of blood lecithin, following insulin administration, when the second blood sample was taken at or about the stage at which convulsions usually occur. There was a decrease of about 5 p.c. in control animals following the injection of the same volume of saline as that of the insulin solution used, and under the same operative technique. This leaves a net decrease of 11 p.c.

Since, however, the lecithin is present, according to Bloor, to a much greater extent in the corpuscles than in the plasma, this fall in lecithin might simply be due to a change in the proportion of corpuscles, and it became necessary to make some blood volume determinations. These have been done on some 25 animals, the hæmoglobin value being taken, in the majority of these cases, as the index of blood dilution. The hæmoglobin was determined in some experiments with Haldane's hæmoglobinometer, and in others by the acid hæmatin method using a colorimeter. Both methods agreed. On an average the blood at the moment of convulsions was diluted some 15 p.c., although the individual variations from this figure are in some cases large. A few hæmatocrit experiments and red cell counts agreed fairly well with the changes in the hæmoglobin values. In these cases sufficient blood was taken (not more than 0.4 c.c. all told) at each interval for a duplicate hæmatocrit, blood count and hæmoglobin determination, but in the other experiments only sufficient blood was taken at each interval for a duplicate hæmoglobin determination, so that the possibility of the dilution being merely due to repeated small hæmorrhages was minimised. Nevertheless control animals, injected with saline in place of insulin solution, but otherwise subjected to exactly the same operative technique as the insulin animals, showed a slight drop in the hæmoglobin, the average fall being 3 p.c.

In one series of experiments in which only two 0.2 c.c. blood samples were taken (one before the administration of insulin, and the second at the onset of convulsions) the same average fall of hæmoglobin was found, namely 15 p.c. calculated on the original hæmoglobin value, as in the experiments just described, in which more frequent blood samples were taken.

The curves below show the results obtained. The rate of descent of the curve varied roughly with the amount of insulin given. In the three cases shown in Fig. 1, in which insulin "Y" was used, 15 rabbit doses were

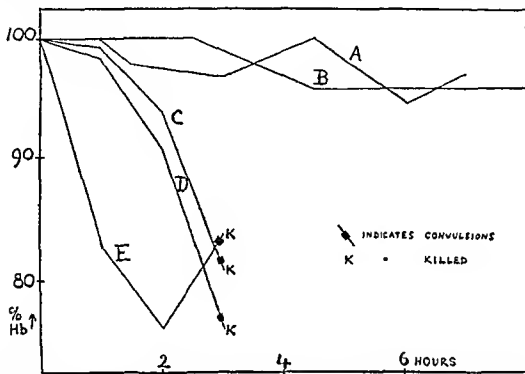


Fig. 1. Effect of insulin on blood volume, showing typical curves with insulin Y (C, D, E). A and B are typical blood concentration curves of controls subjected to the same operative technique, but injecting saline instead of insulin solution. Original hæmoglobin value taken as 100 p.c. in each case.

given, and the fall was very sharp. When however considerably less insulin was given, and the animal began to recover from its effects without having reached the convulsive stage, it was found that a corresponding recovery in the blood volume occurred. This return of blood volume towards the normal took place when glucose was given at the onset of convulsions, if the volume of injected glucose solution was not too large. Examples are seen in Fig. 2. (To avoid confusion, only a small number of typical experiments is given. The initial hæmoglobin value has been taken as 100 p.c. in each case.)

It will be seen that in a number of cases a definite but transient rise was obtained at the outset. This rise was found to follow the use of one make of insulin, insulin "X," or the solid insulin hydrochloride sold by

the same makers, which type of insulin was used in the majority of our experiments, but was not observed in cases in which another brand of

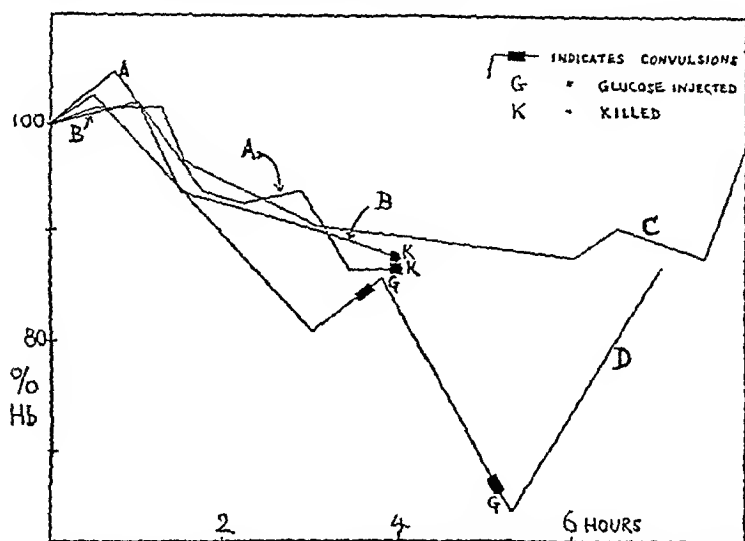


Fig. 2. Effect of insulin on blood volume showing (a) typical effects with insulin X (A and B), and (b) return towards normal after some hours (C and D). Original hæmoglobin value taken as 100 p.c. in each case.

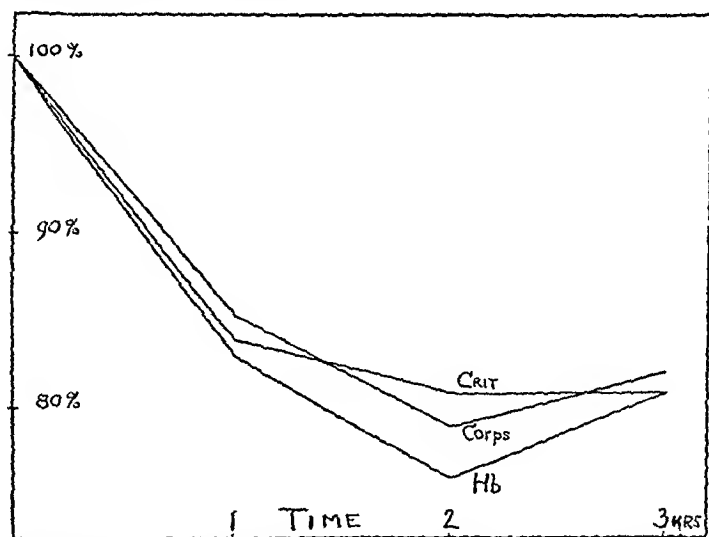


Fig. 3. Hæmoglobin, red corpuscle count, and hæmatocrit varying together in the same animal after insulin; typical curve. Reading at zero hour taken as 100 p.c. in each case.

insulin, insulin "Y," was employed. This may well be correlated with the observation made by other workers that whilst insulin X causes a tem-

porary increase in the blood pressure followed by a fall, there is no initial rise in the case of insulin Y. The temporary increase in blood pressure would probably lead to a short period of increased filtration of liquid from the blood into the tissues before the characteristic insulin effect on blood volume (and blood sugar) had time to develop.

Since there was such a marked change in the blood volume as shown by the hæmoglobin value, corpuscular count and hæmatocrit, it was of some interest to find out whether any measurable change in the osmotic pressure of the serum followed. That this is not the case is clearly shown by the following determinations of the depression of freezing point of the serum:

Control animal	Comparable animal from the same litter after insulin convulsions
·560°	·565°
·590	·565
·572	·585
·574	·575

No anæsthetic was used in any of the foregoing experiments. The results obtained by Drabkin, Page, and Edwards (7) in which they find an increase in the concentration of the blood following the administration of insulin to dogs anæsthetised with iso-amyl ethyl barbituric acid may possibly be due to the combined effect of the anæsthetic and the insulin, though they state that one control experiment indicated that the anæsthesia alone had little effect on either blood sugar or blood concentration. Dale and Laidlaw's (8) work with histamine shows very clearly that the joint effect of anæsthetic and hormone may be widely removed from the sum of their effects when administered separately. The marked diuresis, for example, observed by Drabkin, Page and Edwards, may not be due to insulin. It is also possible that an insulin with a marked pressor action may have been employed, or that the reaction of dogs to insulin, as far as blood volume is concerned, may be quite different from that of rabbits. It is known that dogs are more resistant to insulin convulsions than are rabbits.

On the hypothesis that insulin brings about blood dilution by rendering the kidney temporarily impermeable to liquid while the absorption of liquid from the intestinal tract is still going on at the normal rate, the retention of liquid in the blood during the first two hours after administration of insulin would be insufficient to account for the whole of the blood dilution effect. The average increase in the blood volume is 15 p.c. Taking the blood volume of the 2 kilogram rabbit as 110 c.c.,

the same makers, which type of insulin was used in the majority of our experiments, but was not observed in cases in which another brand of

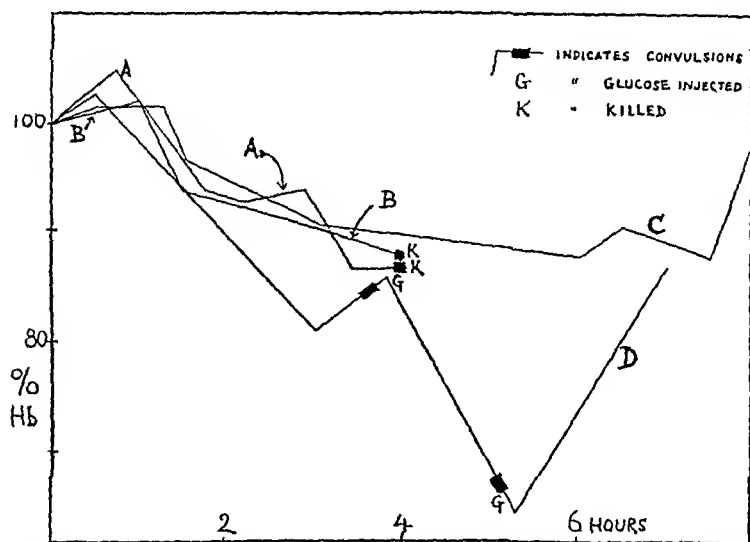


Fig. 2. Effect of insulin on blood volume showing (a) typical effects with insulin X (A and B), and (b) return towards normal after some hours (C and D). Original hæmoglobin value taken as 100 p.c. in each case.

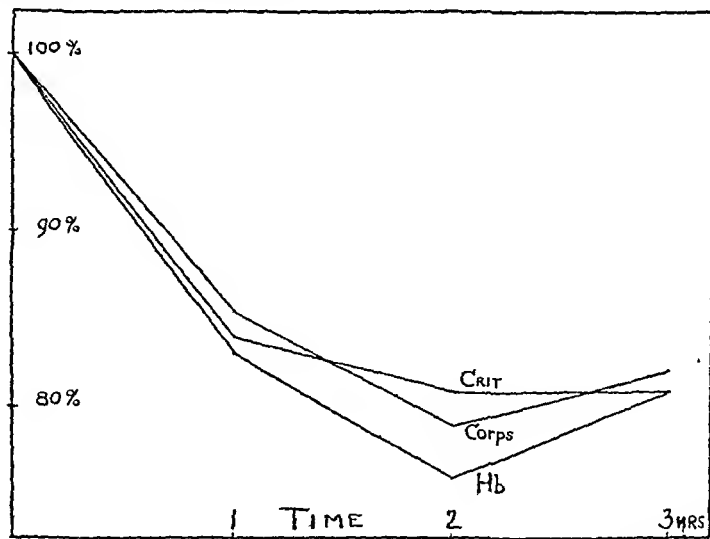


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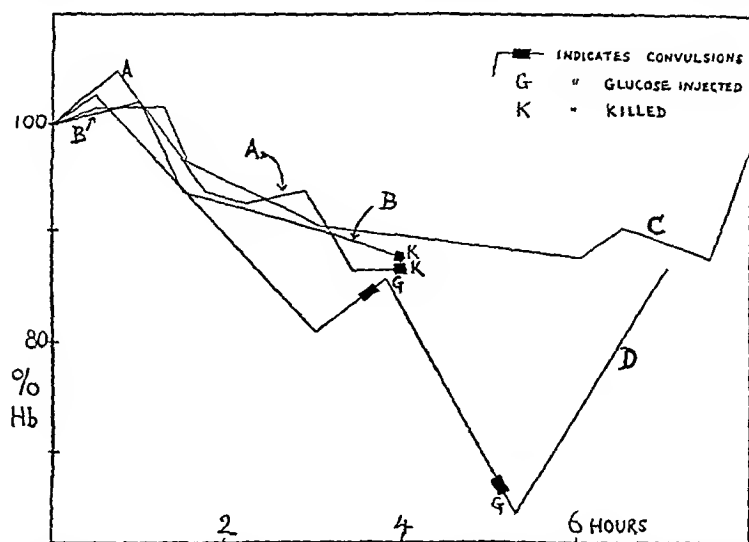


Fig. 2. Effect of insulin on blood volume showing (a) typical effects with insulin X (A and B), and (b) return towards normal after some hours (C and D). Original hæmoglobin value taken as 100 p.c. in each case.

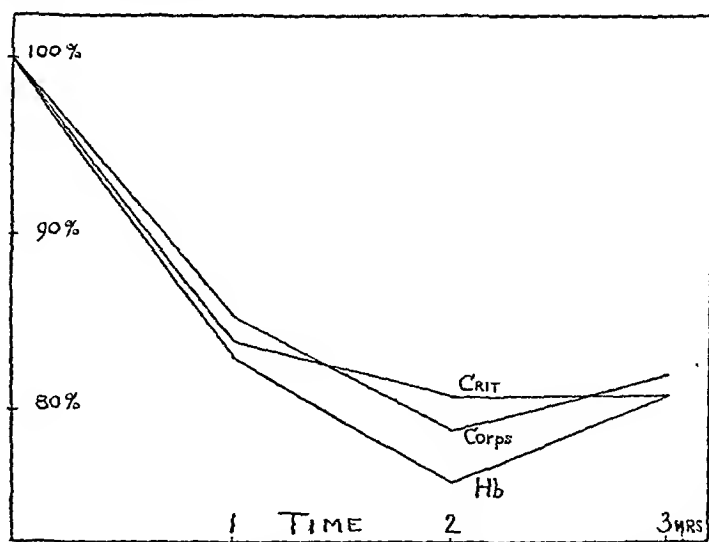


Fig. 3. Hæmoglobin, red corpuscle count, and hæmatocrit varying together in the same animal after insulin; typical curve. Reading at zero hour taken as 100 p.c. in each case.

insulin, insulin "Y," was employed. This may well be correlated with the observation made by other workers that whilst insulin X causes a tem-

determinations in blood before and after the giving of insulin, particularly in the case of substances not equally distributed between the corpuscles and plasma, can only be accepted if this volume change is taken into account.

The osmotic pressure of serum remains the same before and after the injection of insulin.

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THE GLOMERULAR CONTROL OF THE KIDNEY BLOOD FLOW. BY J. M. O'CONNOR.

(From the Physiology Department, University College, Dublin.)

THE pronounced influence of the blood-pressure on the activity of the kidney is a characteristic feature of that organ. Ludwig, believing this influence to be direct, held it a strong argument in favour of the formation of urine by filtration. Heidenhain, on the other hand, thought that the pressure acted indirectly by influencing the quantity of blood flowing through the kidney. Richards and Plant⁽¹⁾ have recently given an interesting account of these opposing views, and with an ingenious technique, by which a constant quantity of blood could be forced through the kidney, have supplied evidence that the pressure, and not the resulting flow, is the essential element in this circulatory influence. Keeping the flow constant, the blood-pressure varied with the quantity of urine. They thus confirm Ludwig's original view and appear to disprove Heidenhain's explanation.

A consideration of the anatomy of the kidney shows, however, that the dependence on blood-pressure may be given a different significance reconciling it with a purely secretory theory. The flow of urine along a uriniferous tubule requires a head of pressure. This pressure must be a maximum at the top of the tubule in the capsule of Bowman. The capsule almost completely surrounds the glomerulus. If consequently as a result of an increased secretion pressure or a fall of blood-pressure the pressure of the urine in the capsule becomes higher than the pressure of the blood in the glomerular capillaries these will be completely closed. This will result in the blood supply through the efferent vessel of the glomeruli being cut off and any formation of urine not only from the glomerulus but also in the tubule will cease until the pressure of the urine within them has fallen sufficiently to permit the re-opening of the glomerular vessels. The driving head of pressure and consequently the flow of the urine would be limited by the glomerular pressure even if filtration plays no part in the process.

The laws governing the flow of fluids in elastic tubes have not been examined. Professor A. W. Conway, whom I must thank for the trouble he has taken, informs me that the expression governing the

relation between pressure and flow in elastic tubes will be approximately of the following form;

$$F = F_0(1 + KP),$$

where F is the quantity flowing in unit time, F_0 the flow according to Poiseuille's law, P the pressure head and K a constant depending in part on the elasticity of tube. Values permitting the application of this law are not available, but there is no objection to applying Poiseuille's law directly to determining the pressure prevailing under steady conditions, as has been done by Brodie(2), provided that reliable values can be got. In the case of the rabbit approximate calculations on probable values from previous observations(3), making the arbitrary assumption that the urine comes altogether from the glomerulus and that there is no absorption, give a pressure of the urine in the capsule as 10 mm. Hg above that in the collecting tubules. In this calculation the bore of the tubule was taken as 10μ if it were really 8μ —and it is not possible to be confident in such measurements—the pressure difference would be 25 mm. Hg. No significance can be attached to such calculations, but they show that there is nothing improbable in thinking that the pressure of the urine in the capsule of Bowman may approximate to the capillary blood-pressure in the glomerular loops, which itself has not been definitely determined. If the pressure of the urine goes above the pressure of the blood in the glomeruli these soft structures would, so far as one can judge, offer no resistance to the compressing force.

The suggestion put forward is, then, that this theoretically possible play of urine pressure on blood flow through the kidney is of functional significance; that urine is formed by a process of secretion, and that one function at least of the glomerulus is to modulate the blood flow and consequently the quantity of urine formed from it in accordance with the momentarily prevailing distension of the tubules and ducts. In the absence of some such mechanism a sudden increase in the flow of urine as the result of a diuretic stimulus might cause an injurious distension of the higher portion of the tubules. At this point attention may be called to the fact that according to the reconstruction of Huber(4) not only the proximal but also the distal convoluted tubule coil is in the immediate neighbourhood of the glomeruli from which it originates, and consequently that the blood supply of each tubule, except for the loop portion, passes to a very large extent through the corresponding glomerulus.

According to this theory, there would be in many cases no great difference between the pressures on opposite sides of the glomerular

membrane, and consequently a fall of arterial pressure would cause a disturbance of this balance which would show itself in the blood flow through the kidney. Experiments have been done on this, and in considering them the assumption will be made for simplicity of expression that urine is formed purely by secretion. After the evidence has been presented the justification for this assumption in the light of the results obtained will be examined.

The following was the experimental procedure. The animals (rabbits) were anaesthetised with urethane. The intestines from the pylorus down were removed; a cannula introduced into the left ureter; a loop passed round the vena cava just above the left renal vein and the abdominal aorta ligatured low down. All side branches into the cava, from the renal vein down, were tied and a cannula sealed on to a large glass tube introduced upwards into the cava. This "bleeding tube" was previously well coated with paraffin wax. From a stopper in the upper end of the "bleeding tube" a connection was made through a T-tube with a bellows recorder. The third limb of this T-tube was attached to a glass tube the opening of which was held suspended by a weight over a trough of mercury. By pressing a lever the tube was dipped into the mercury, thus sealing off the connection between the bellows and the bleeding tube from the atmosphere, while at the same time a stop-watch was started to show the time during which the side tube was closed. Thus by keeping the lever pressed down for the time indicated by the watch, the quantity of blood entering the bleeding tube during this period was recorded on a moving drum. To take an observation the clip on the cava below the renal vein was opened and the loop above tightened. The blood from the left kidney then flowed into the tube and the blood-pressure fell. The bleeding tube was placed as horizontally as possible so that the increase in venous pressure when the tube was filled was not more than 2mm. Hg. While the bleeding tube was filling the side tube of the bellows recorder was closed for a number of successive periods of 10 secs., the interval between each observation during which the side tube was open being usually no longer than sufficient to permit the recorder to return to its base line. Simultaneously records of the arterial pressure and the falling drops of urine were recorded. The heights of the outflow records were subsequently accurately measured and compared with the corresponding mean blood-pressures. At the end of an observation on a suitable readjustment of clips the blood was blown back into the circulation. In many cases as a matter of convenience the renal flow included also that from the left suprarenal and accompanying lumbar vein. Preparatory

injections of saline and 5 p.c. glucose were given when necessary to increase the blood-pressure or produce a flow of urine.

Before passing on to the results with the secreting kidney it is first necessary to consider how the flow of blood commonly varies with the blood-pressure. This was tested by an obvious rearrangement of the procedure described above, in which the blood flow from the hind limbs and lumbar region was directed out into a bleeding tube introduced into the left renal vein. As may be seen from Fig. 1 the curve is for a great

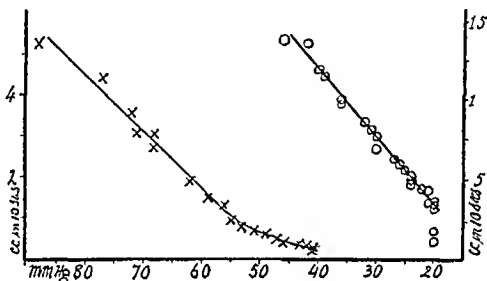


Fig. 1. On left, blood flow through hind limbs, etc. Ordinates on left. On right, blood flow through kidney not secreting (renal nerves cut). Ordinates on right.

part of its course indistinguishable from a straight line. It may be incidentally noticed that the blood flow becomes relatively negligible at quite high arterial pressures.

It is to be noted that as the terminal pressure rose, though but slightly, owing to the filling of the bleeding tube, while the arterial pressure fell, the pressures, marked out on this and subsequent graphs, do not give the true change in the head of pressure. If allowance were made for this, the straight portion would become slightly more convex towards the abscissa, as an application of Professor Conway's formula (for it is true uniform pressure) to the circulation would require. In some cases the convexity is distinct without any such allowance having been made (Fig. 3).

We may now turn to consider how we might expect the graph of pressure and flow through the kidney to be modified if the expectations put forward be realised. There are three possibilities.

If there be no formation of urine, an interference due to pressure of the urine on the glomeruli would not be expected. This seems to be realised as in Fig. 1. Attention may here be called to the contrast between the graph of kidney flow and hind limb flow. The straight line portion of the graph tends to cut the abscissa at much lower pressures. In other experiments, however, in which without a mechanical obstruc-

tion no urine emerged from the cannula, a different result was obtained. There was during a portion of the graph a relative increase in blood flow with falling pressure. This might be explained in the following way. The kidney is being stimulated to secrete, but in the steady state the pressure in glomeruli is so low that the blood supply becomes cut off before the head of pressure rises sufficiently high to force out the urine at an appreciable rate.

The second case to be considered is one in which the ureter is obstructed. Here one would expect the pressure of the urine to have risen to such a height that the glomeruli would all be closed. In fact one finds during a portion of the range of the graph that the blood flow rises, at least relatively, with falling blood-pressure. In this, as in the previous case, the explanation offered is that with the fall in tension in the arteries the kidney tubules are able to expand more freely, and in spite of the falling capillary blood-pressure, permit the glomeruli to open, giving free paths to the previously restricted flow.

The third case is where there is no obstruction to the flow of urine. One would under steady conditions expect all the glomeruli to be open and the only possible change would be a sudden diminution of the capillary blood-pressure below the urine pressure, with a consequent sudden diminution of blood flow. This is the common result and is shown in Fig. 2 in which the blood-pressure fell steadily and the outflow for the

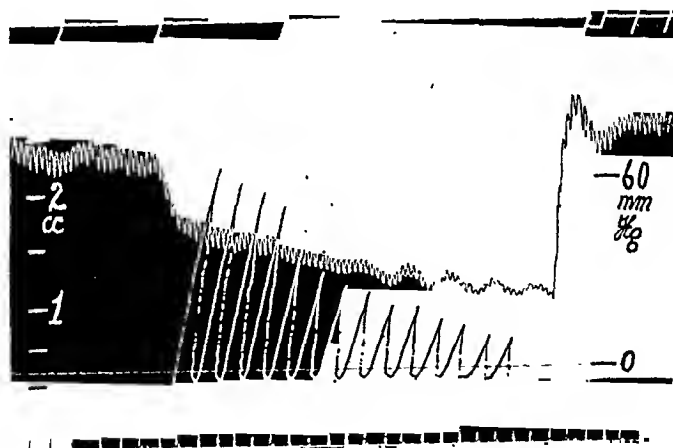


Fig. 2. Record of blood flow in 10-second periods in an active kidney. There are recorded from above downwards: drops of urine; blood-pressure; quantity of blood flowing in successive periods of 10 seconds; and the time in 10 seconds.

first four 10-second periods fell uniformly, the 5th however drops suddenly, and only from the 7th onwards does the fall in flow accompanying the fall in pressure again become uniform. Occasionally, experiments are met with in which the kink, instead of being negative, as in this case, is of a positive kind. Some of these are no doubt due to partial obstruction at the cannula. The experiment given in Fig 3 illustrates this. During the first observation the urine was flowing through the cannula but the ureter was visibly distended—a “positive” kink is recorded. Before the subsequent observation the ureter was cut open—a marked “negative” kink is visible. All the experiments do not seem to be met by this

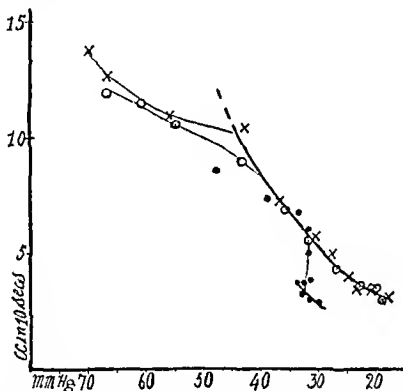


Fig 3 Renal nerves cut. Crosses and white circles, observations with obstructed ureter. Initial B P, 90 and 78 mm Hg respectively. Black circles, ureter cut open, B P 56 mm.

explanation. However, according to the theory advanced, it is possible that the balance between opening and closing of the glomeruli is a delicate one and the fall of blood-pressure so alters the state of mechanical tension within the fibrous capsule of the kidney that in one case uriniferous tubules are allowed freer expansion, the pressure within them falls and some previously closed glomeruli open, whereas, in the other cases glomeruli merely close as the result of the fall in blood-pressure being more pronounced than the fall in urine pressure.

Occasionally both varieties of kink are met with during the one out-flow observation. The experiment illustrated in Fig 4 is an example. In this the blood-pressure which had been 91 mm Hg had fallen to 76

before the first 10-second period was recorded. There was then a slight rise of pressure but the blood flow was smaller and the third and fourth

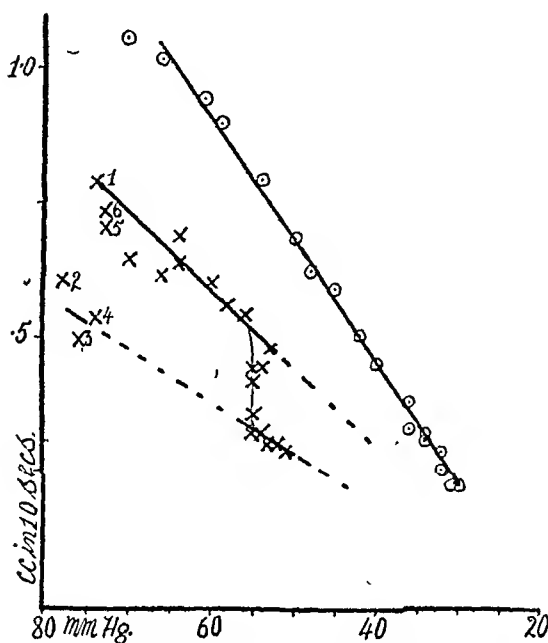


Fig. 4. See text. First observation, crosses; second observation, circles.

10-second periods corresponded with this. It is suggested that the glomerular blood-pressure had just at this point fallen below the urinary pressure. In most experiments the blood flow would now follow the lower line but here the flow rose again to the old level. It is suggested that the tubules had in the meantime expanded and the pressure of the urine fallen below the pressure of the blood with the result that the glomeruli re-opened. The flow graph now proceeds to follow along a straight line until a pressure of about 55 mm. Hg is reached, when there is again a vertical drop in the graph due, it is suggested, to a renewed collapse of the glomeruli. Further points now lie along another straight line at a lower level but similar to the previous one. This line, if produced back, cuts the group formed by the second, third and fourth points. Whether the explanation is correct or not, it seems that a resistance to the blood flow, which was thrown in for the second, third and fourth points and then disappears, is again thrown in towards the end of the observation. This particular experiment has a further interest. The blood was forced back into the circulation but the urine which had been flowing at a drop (*circa* 30 c.mm.) in 30 secs. practically ceased

(1 drop in 200 secs.). Another blood flow observation taken within $3\frac{1}{2}$ minutes of the first gave a straight line graph which can be correlated with the absence of urine formation.

It can be objected that the first six points on the first of these two graphs occur with but very minute changes in pressure and that it is difficult to imagine the changes described in such a narrow range. Against this, however, it must be remembered that the initial blood-pressure was higher than the first recorded on the graph and that six observations occupied slightly over a minute during which the changes described going at different rates might well occur. A further objection against not only this but all similar experiments is that the kinks described might well be due to vasomotor reactions, particularly vasomotor reactions against hæmorrhage. Although it is possible to argue against this, it is obviously better to rely on experiments in which the kidney has been denervated (Figs. 1, 3, 5).

Vasomotor reactions against hæmorrhage are commonly believed to occur, but the evidence for them does not seem to be extensive (5, 6).

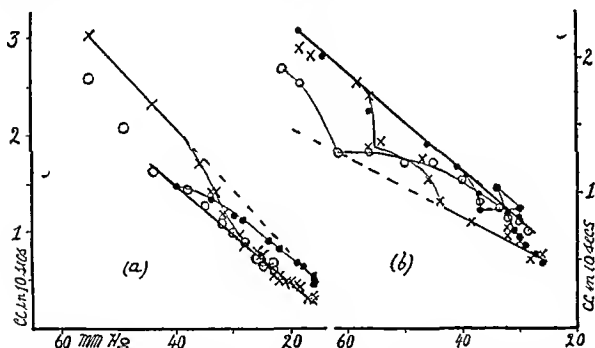


Fig. 5. (a) Observations on kidney with renal nerves cut. Ordinates on left.
 Black circles... no urino Initial blood-pressure, 58 mm. Hg.
 White " ... 1 drop in 90 secs. " " 104 "
 Crosses ... 1 " 60 " " " 87 "
 Observations taken during $\frac{1}{2}$ hour. 80 c.c. saline between 1 and 2.

(b) Observations on kidney with renal nerves cut and suprarenals removed. Ordinates on right.

Crosses ... 1 drop in 20 secs. Initial blood-pressure, 92 mm. Hg.
 White circles... " 10 " " " 80 "
 Black " ... " 20 " " " 78 "

Observations taken in 20 minutes.

Fig. 5 shows the results obtained (a) with a kidney in which the nerves running in the pedicle have been cut; (b) in an animal in which in addition to nerve section both suprarenals had been ligatured off. Taking experiment (a), it will be noticed that the points of each observation sway between two straight lines. In the first observation urine was not flowing, and on fall of pressure the flow passed from the line of low flow to the line of larger flow. This has been already explained as being due to the pressure of the urine being sufficiently high to keep the glomeruli closed but not sufficiently high to force out urine at an appreciable rate. In the other observations in both of which urine was being secreted, we find the pressure-flow graph swaying from the high line on to the low. That is the same resistance which in the previous case was shown being removed is now shown being put in. In the third case the flow record has apparently reached the low line before points could be recorded. We have then this graph explicable by the throwing in or out of the same resistance in three observations, a resistance not due to any stimuli passing through the kidney nerves.

In the second set of observations on Fig. 5 not only were the nerves cut but the suprarenals ligatured off. Here any individual set of observations tends to sway somewhat irregularly, but taken all together they can be seen to be explicable by the same resistance being introduced or removed at various blood-pressures, so that the beginnings of the graphs in two cases, and the ends in all, lie along two straight lines.

In these experiments, and others of a similar kind, the kink is not to be explained by any interference through the renal nerves or by a secretion of adrenaline from the suprarenals. The fact that the different observations in one experiment overlap to the extent described—an overlapping which, as can be seen from Fig. 4, is not to be got where the nerves are not cut—seems to confirm the natural expectation that extra-renal influences in the intervals between the observations have not affected the kidney circulation, and the sudden changes in the blood flow during the observations are scarcely attributable to such external influences. It can none the less be legitimately suggested that cutting the kidney nerves and ligaturing the suprarenals is not sufficient to completely isolate the kidney. Nerve impulses might pass through the vessel walls or some less obvious hormonal mechanism be excited to activity by the fall of blood-pressure.

To meet this objection the following experiment was done (Fig 6). A maximal vaso-constriction was produced by a continuous infusion of 3 c.c. adrenalin 1 : 50,000 per minute, immediately before and during

the observation of the blood flow The blood-pressure rose from 96 mm to 186 mm on starting the injection The record of flow then taken shows a positive kink That is, there is a sudden diminution of resistance to blood flow although the vessels were maintained in a state of maximal constriction

In the experiments considered so far an effort has been made to disturb the presumed balance of pressure between urine and blood at

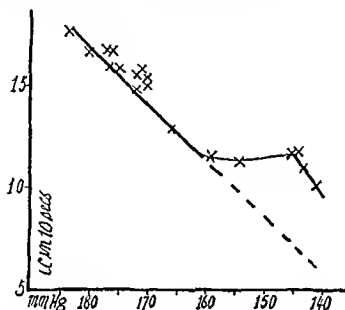


Fig 6 Original B P 96 mm Hg 1 drop urine in 10 secs Continuous infusion of 1 50,000 adrenalin 3 c c per min , rate of urine falls 80 secs later B P 186 mm Hg record of flow started

the glomerulus by lowering the blood-pressure Observations were also made in which it was attempted to disturb the balance by increasing the pressure of the urine by forcing fluid under pressure into the ureter In fact decided falls of flow disappearing on removal of the pressure were produced, confirming results obtained by others, such as Burton-Opitz and Lucas(7) Apart from the difficulty or impossibility of forcing fluid back into tubules from the ureter, these observations are vitiated by the rise in pressure in ureter presumably pressing on the veins In fact, the diminutions of flow produced are usually very much greater than those produced in experiments previously alluded to, and are in part, if not altogether, to be explained as venous obstructions These experiments need not be considered in detail

On the secretion theory of urine formation there is another method by which these pressure interactions in the kidney might conceivably be disturbed If, as the result of some diuretic stimulus, a sudden increase in the secretion of urine occurs, a brief time will elapse before the tubules and ducts have sufficiently expanded to allow a free outflow

pressure of tubules on veins lying between them, but rather to be due to some essential feature of the vascular arrangement such as the glomeruli.

If, however, this argument be held sound another point arises. According to the observations of Huber(s), practically all the blood of the kidney, cortex and medulla, passes through the glomeruli. But in none of the experiments has the obstruction to blood flow found been even approximately complete. This, however, would be explained by the commonly held view that only a fraction of the tubules are active at one time and a quantity of blood flows without hindrance through the glomeruli of quiescent tubules.

If the experiments are to be explained on the lines suggested it is yet in any sense proved that this mechanism is of significance. At there is a positive pressure in the glomerular capillaries and a positive pressure of the urine in the capsule is certain. Further, it seems from the anatomical arrangement that if the pressure of the blood goes above the glomerular blood-pressure the blood flow must be stopped. The experiments demonstrating a negative kink show, subject to criticism, that this does occur when the blood-pressure falls. This is hardly reconcilable with the view that the pressure in the tubules is due merely to filtered blood plasma. Although the experiments showing a "positive" kink are not to be explained so easily, the attribution of a functional significance to the anatomical relation is a surmise. The results at least suggest further experiments, and the theory on which they are based may be the true explanation of the dependence of the activity of the kidney on the pressure of the blood.

In fact decision my thanks are due to the Editor for criticism. were produced, *Continued*.

Opitz and Lucas(7). *Appl. Phys.* SUMMARY.

forcing fluid back into tubules from structure of the kidney a secretion vitiated by the rise in pressure in ureter. A uriniferous tubule could not go to veins. In fact, the diminutions of flow pressure in the capillaries of the Malpighian tuft are greater than those produced in experiments on the kidney tubules. This is in part, if not altogether, to be explained by the dependence of the secretion on the pressure of the blood. These experiments need not be considered in detail.

On the secretion theory of urine formation through the kidney might be by which these pressure interactions in the kidney fail. An arrangement be disturbed. If, as the result of some diuretic during a fall of blood-pressure, in the secretion of urine occurs, a brief time the tubules and ducts have sufficiently expanded to

3. Observations on the hind limbs show that the graph of blood-pressure and blood flow is for a great part of its course practically a straight line.

4. Observations on the kidney show that in these a similar graph is broken by decreases or increases in the blood flow. Such kinks can be seen when the renal nerves are cut and the suprarenals ligatured off, and are explicable on the postulated obstruction to the blood flow at the glomerulus.

5. The bearing of these results on the theory of urine formation is discussed.

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ON THE CONSTANCY OF THE BASAL METABOLISM.

By GRAHAM LUSK AND E. F. DU BOIS.

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I. Basal metabolism in dogs.

It has been observed that the basal metabolism of a dog (No. XIX) placed in a calorimeter may remain so constant that during periods of several months in two successive years the variation (± 2.9 p.c.) in seventeen experiments was scarcely greater than the error (± 1.9 p.c.) in eight determinations of the consumption of oxygen when the calorimeter was tested for accuracy by burning alcohol within it. In this animal, as in others reported in this series (1), the basal metabolism was always higher when it returned from the country in the autumn after an active life in sunshine and fresh air. The influence of "cage life" in a darkened room will each year reduce the basal metabolism from a level of 20 calories to one of 16.5 calories per hour. Another factor, and one which tends to increase the oxidative level, is that due to the retention of "deposit protein" after a large ingestion of meat (2).

In a previous publication (3) it has been pointed out that Dogs I and II and a small dog (4.6 kg.) of Rubner's produced respectively 759, 784 and 749 calories per day per square metre of body surface when the formula of Meeh, $\text{Surface} = 11.2 \sqrt{\text{Weight}^2}$, was employed in the calculation. The exact validity of Meeh's formula is doubtful but need not here be discussed.

The accumulation of additional data has gone on. For example, 197 calorimeter experiments have been performed upon Dog XIX during five successive years. It seems justifiable to analyse the data at hand in order to determine whether the basal metabolism of a dog can be approximately measured from the formula of Meeh. Table I presents the results obtained from eleven female dogs.

It appears from these results that the average basal heat production is 772 calories per square metre of body surface per day and that this

TABLE I. The basal metabolisms of eleven well trained female dogs.

Dog	Date	Weight kg.	Sq. metre surface $11.2 \sqrt[3]{Wt.^2}$	Calories			Variation from the average normal %
				Per hour	Per day	Per sq. metre surface per day	
I	1911	15.8	.705	22.3	535	759	- 2
II	1912	9.3	.500	16.2	389	785	+ 1
III	1914	12.5	.603	16.7	401	664	-14
IV	1914	12.7	.610	20.2	485	795	+ 2
XIV	1914	13.1	.622	21.5	516	829	+ 7
	1915	14.6	.669	22.7	532	814	+ 5
XV	1917	9.1	.488	17.2	413	845	+ 9
XVI	1917	10.6	.540	20.0	480	888	+15
XVII	1918	14.9	.678	21.0	504	743	- 4
XVIII	1919	10.8	.547	16.6	398	728	- 6
	1921	10.8	.547	16.6	398	728	- 6
XIX	1920	9.9	.516	17.6	422	818	+ 5
	1921	9.4	.499	17.5	420	842	+ 8
	1922*						
	1923	11.5	.571	16.5	396	694	-10
	1924	11.5	.571	16.5	396	694	-10
XX	1921	9.1	.488	14.9	358	<u>734</u>	- 5
						772	

* Dosage with morphine, and high protein ingestion.

is accurate within an error limit of ± 15 p.c. Using this figure, the basal metabolisms of dogs of different weights would therefore be:

Weight kg.	Surface sq. m.	Basal metabolism, 24 hours Calories		
		Per sq. m.	Total	Per kg.
5	.328	772	253	51
10	.520	772	401	40
15	.680	772	526	35
20	.825	772	637	32

II. Basal metabolism in men.

The basal metabolism of Zuntz (4) was recorded at intervals over a period of 29 years. Using Meeh's formula, it presented 804 calories per square metre of surface per day in 1888 when Zuntz was 41 years old and 792 calories in 1910 when he was 63 years of age. There was no change until later when under-nutrition during the war reduced the rate of the oxidation processes.

Table II presents the record of the basal metabolism of E. F. Du Bois, most of the determinations having been made in the calorimeter of the Russell Sage Institute.

During a period of 11 years twelve different observations of the basal metabolism show an average of 37.7 calories per hour per square metre of body surface (Du Bois formula), the variation being ± 7.6 p.c. from the average.

TABLE II Basal metabolism of E F Du Bois (Height, 178-178.8 cm)

Date	Year	Age Years	Weight kg	Surface Ht Wt sq m	Calories		Variation from average p c
					Per hour	Per sq m per hour	
Mar 13	1913	30	73.6	1.91	77.6	40.6	+7.0
May 17	1913	—	75.5	1.95	73.2	38.1	+1.1
Mar 30	1914	31	74.3	1.93	74.1	38.4	+1.8
May 18	1914	—	73.7	1.92	71.3	37.2	-1.3
" 0	1915	32	74.6	1.93	71.8	37.2	-1.3
" 7	1915	—	74.2	1.93	68.6	35.5	-5.8
Apr 12	1916	33	76.5	1.94	75.4	38.8	+4.0
" 25*	1916	—	77.3	1.95	73.2	37.5	-0.5
Dec 18	1916	34	73.9	1.90	76.2	40.1	+6.3
May 10*	1922	39	78.0	1.97	68.6	34.8	-7.6
Oct 7	1923	41	74.7	1.93	70.8	36.7	-1.8
Apr 10†	1924	—	75.0	1.94	71.7	37.0	-1.8
						37.7	

* 1 hour experiment

† Benedict Roth apparatus

Experiments (Table III) on Lusk are fewer in number. During the summer seasons of 1910 and 1913 the subject was accustomed to take vigorous exercise. For at least 18 months prior to the determination of the basal metabolism in April, 1924, the subject had taken no strenuous exercise and had spent much of the winter in reading and writing at his desk. It is a moot question whether the drastic decline in the basal metabolism is due to advancing age or to the same conditions presented by "cage life" in the dog, as already described.

TABLE III Basal metabolism of G L (Height, 175.6 cm)

Date	Year	Age years	Weight kg	Surface Ht Wt sq m	Calories		Variation from average normal (Sage standards) p c
					Per hour	Per sq m per hour	
Oct. 14*	1910	44	76.0	1.92	82.1	42.8	+7
Nov 3†	1913	47	78.4	1.95	80.9	41.4	+4
Apr 22†	1924	58	78.0	1.94	63.4	32.7	-13

* Benedict respiration apparatus

† Sage calorimeter.

SUMMARY.

1. From the records of experiments with eleven dogs the basal metabolism may be approximately determined within an error limit of ± 15 p c.

2. The same dog may manifest a constant level of basal metabolism extending over two years.

3. A man between thirty and forty years of age may show a basal metabolism during eleven years within a variation of ± 7.6 p.c.

4. The influence of "cage life" in reducing the basal metabolism in the dog has a probable counterpart in lack of exercise and indoor confinement in man.

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THE EFFECT OF VARIATIONS IN BLOOD-PRESSURE
ON PULSE WAVE VELOCITY IN THE BRACHIAL
ARTERY IN MAN. By SYLVIA K. HICKSON¹
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PULSE wave velocity, as measured by means of the hot wire sphygmograph, has been shown to vary with the pressure within the artery, and with the extensibility of the arterial wall in experiments performed upon the excised carotid artery from animals and human subjects(2,4). By means of a hot wire sphygmograph similar to that used for experiments on the excised artery, the pulse wave velocity can be ascertained in the living subject(6), and it has been shown that the velocity increases with advancing age, closely following the rise in blood-pressure which occurs as one grows older(1). In cases where the extensibility of the arteries is identical one would expect to find a rise in pulse wave velocity with a rise in blood-pressure, as the velocity appears to vary with the pressure within the walls, apart from the consideration of external factors, such as arterial contraction.

The effect of variation of the blood-pressure upon the extensibility of an artery in the living subject has been worked out previously(3) by compressing a known length of the artery under a sphygmomanometer bandage at different pressures and estimating the pulse wave velocity in each case. This yielded a series of results showing that the increased pressure was accompanied by an increase of velocity in the affected part; but since the whole of the arm under the bandage was subjected to pressure, it was thought possible that factors, other than pressure changes within the artery, might have contributed to this result. To eliminate these external factors and to obtain a localised change of blood-pressure in one artery, our subject has remained in the same position throughout the experiment, the right arm being passively raised or lowered. In all cases we found a fall in systolic blood-pressure of 20 to 30 mm. Hg, when the arm was raised above the head from the horizontal position. The blood-pressure was read also in the left arm, first when both arms were horizontal, and secondly with the left arm in

¹ Working for the Medical Research Council.

the same position after the right arm had been raised to the vertical position. The blood-pressure in the left arm did not vary whatever position the right arm assumed.

It has been shown previously (5) that the pulse wave velocity varies with respiratory changes, the velocity being higher during expiration than inspiration, corresponding with the higher blood-pressure in the peripheral arteries. These measurements agree with the majority of those tabulated by Wiggers (7). To obtain comparable points for measurement in similar phases of respiration, a respiratory curve was taken from the subject simultaneously with the carotid radial tracings.

Method. In making these investigations we have worked with normal subjects between the ages of 20 and 30, and have estimated the pulse wave velocity by means of two hot wire sphygmographs, the one connected with the carotid artery by means of a cup-shaped receiver, and the other by the armlet of a Pachon oscillometer to the radial artery at the wrist. The instrument, which has been fully described in a previous paper (1), consists of a Wheatstone's bridge, the hot wire forming one of the arms. The sphygmographs were connected with fine copper fibres, soldered between the poles of an Einthoven string galvanometer, the deflections of which, recording simultaneous carotid and radial pulsations were photographed on long strips of bromide paper. To record the respiratory curve an apex beat tambour was applied to the chest wall by an elastic bandage, and connected by rubber tubing to a second tambour with a light lever placed in the beam of light from the galvanometer. Movements of the chest wall caused a deflection of the writing point, the movements of the shadow being recorded on the same strip

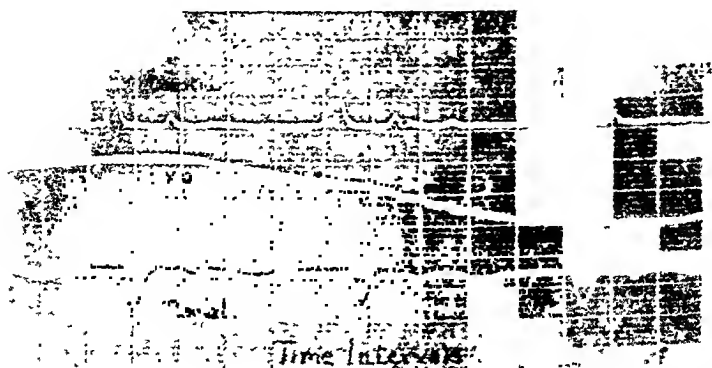


Fig. 1. Read from left to right. Curves from above downward—carotid artery, respiration, radial artery. Time in 0.2 sec.

of paper as the carotid radial tracing. A time marker cutting off the light every 0.2 sec. was employed.

The blood-pressure having been determined by the auscultatory method, over the brachial artery at the bend of the elbow, with the arm in the vertical position, records were taken of the carotid and radial pulsations. The right arm was then supported vertically upwards, to eliminate the effect of extraneous muscular contraction on the artery, and the blood-pressure was again read in a similar manner. A second record of the carotid and radial deflections was then obtained. The distance from the centre of the cup on the carotid artery to the right sterno-clavicular joint was measured, together with the length of the radial and brachial arteries from the proximal border of the radial bandage to the sterno-clavicular joint, the difference between the two being the distance traversed by the pulse wave in the time between the carotid and radial oscillations on the record.

The long paper records were measured in two ways: (a) by means of a Lucas Comparator, (b) with a glass ruler etched in millimetres. The Lucas Comparator is the more accurate method, but the majority of our measurements were taken with the glass ruler as being sufficiently accurate for the purpose. The calculation was estimated by the time lines occurring on the record every 0.2 sec., an accuracy of .001 sec. being obtained.

Carotid-radial intervals were taken at similar points on the respiratory curve, and all these deflections were measured unless malformation of the curve was evident.

Exp.	Distance in cms.	Arm down			Arm up			Differ- ence in vel. m. per sec.
		B.P. in mm. Hg	Time in secs.	Velocity in metres per sec.	B.P. in mm. Hg	Time in secs.	Velocity in metres per sec.	
1	52	113/65	Insp. .0953 Exp. .0835	5.5 5.9	98/60	Insp. .1153 Exp. .1080	4.5 4.8	1.0 1.1
2	51.5	140/65	Insp. .0972 Exp. .0953	5.3 5.4	115/60	Insp. .1375 Exp. .1334	3.7 3.9	1.6 1.5
3	47.5	130/80	Insp. .0772 Exp. .0720	6.2 6.6	100/55	Insp. .1115 Exp. .1030	4.3 4.6	1.9 2.0
4	50.5	110/70	Insp. .0943 Exp. .0902	5.4 5.6	85/55	Insp. .1373 Exp. .1329	3.7 3.8	1.7 1.8
5	50	115/75	Insp. .1170 Exp. .1115	4.3 4.5	84/45	Insp. .1450 Exp. .1332	3.4 3.8	.9 .7
6	51	120/70	Insp. .0816 Exp. .0782	6.2 6.5	100/55	Insp. .1124 Exp. .1001	4.5 5.1	1.7 1.4

The figures given for time in this table are the mean of the four or five time intervals which were measured in each case.

The velocity was worked out by estimating d/t where d is the distance between the length of the radial-brachial and carotid arteries in centimetres and t the time interval between the upstrokes of the carotid and radial deflections in seconds. The velocity was estimated in metres per second.

CONCLUSIONS.

In all our cases where a fall in blood-pressure was noted on raising the arm to a vertical position, the lengthening of the carotid-radial time interval was observed, with an appreciable slowing of the pulse wave velocity, as will be seen from the table. This postural variation in blood-pressure is considerable and must be accounted for when estimating the pulse wave velocity in arteries such as the femoral. In timing the arrival of the pulse wave at the wrist, asynchrony may be found if there is a difference between the position of the arms, the fact being of importance in certain clinical cases, such as aneurysm of the arch of the aorta. Thus asynchrony of the two pulses in cases where the external conditions are identical, indicates alteration in pressure in one artery, with delayed transmission of the pulse wave.

These variations in pulse wave velocity with small changes of blood-pressure are important as indications that constant modification of blood-pressure is occurring independently of permanent alteration of arterial elasticity.

The Expenses of this investigation were in part defrayed out of a grant from the Government Grants Committee of the Royal Society to one of us (B. A. McS.).

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A COMPARISON OF BLOOD CURVES CONSTRUCTED WITH ARTERIAL AND WITH VENOUS BLOOD.

BY F. R. FRASER, G. GRAHAM AND R. HILTON¹.

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IN the course of observations on clinical cases of dyspnoea we have used two methods for determining the CO_2 tension of the arterial blood: (1) by interpolation of the arterial CO_2 volume on the CO_2 dissociation curve, and (2) by interpolation of the $p\text{H}$ of the arterial blood on the $p\text{H}$ - CO_2 tension curve, using the Dale-Evans⁽³⁾ method for the direct determination of $p\text{H}$. If venous blood from an arm vein was used for the construction of the curves, the figures obtained for the CO_2 tension in the arterial blood by the two methods differed very considerably as a rule, sometimes by as much as 20 mm. of Hg; but if arterial blood was used, the figures obtained by the two methods agreed, or differed only by 3-5 mm. of Hg. This suggested that the curves obtained with venous blood, although this was fully oxygenated, were not identical with those obtained with arterial blood. It was therefore decided to compare the curves obtained from the same individual, using venous blood from an arm vein and arterial blood. For this purpose it was necessary to obtain 40-50 c.c. of arterial blood with certainty, and by puncturing the femoral artery we were able to do this (7).

Method. 50 c.c. of arterial blood were drawn into a syringe containing 0.25 gm. of potassium oxalate and enough liquid paraffin to obliterate the air space, and immediately transferred under paraffin to a glass tube surrounded by ice. Venous blood was drawn in the same way from an arm vein, using as little compression as possible. Sometimes no compression was needed; in some cases a momentary compression was necessary until the needle was in the vein, but was released before the blood was drawn; in other cases compression was needed throughout the withdrawal of the blood.

Samples of the arterial blood were taken for dialysis and $p\text{H}$ determination by the Dale-Evans method, and other samples were taken for the determination of CO_2 volume, using the Van Slyke gas analysis

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apparatus(14). Four 8 c.c. samples of arterial, and four of venous blood, were placed in tonometers of 450 c.c. capacity filled with different mixtures of CO_2 and air, and equilibrated at 37.5°C . for 15 minutes in a water bath. The blood samples were thus fully oxygenated, but exposed to varying partial pressures of CO_2 . Only two tonometers could be accommodated in the bath at one time, so that this procedure occupied 1-1½ hours. The original blood was kept throughout the experiment on ice, and the samples drawn off immediately before analysis or transference to the tonometers. At the end of 15 minutes in the water bath, the blood in the tonometers was allowed to flow out under paraffin and again kept on ice till required for the determination of the pH and CO_2 volume. The gaseous pressures of the contents of the tonometers were determined while the tonometers were still in the bath, and the contents were finally analysed and the partial pressures of CO_2 determined, using the Haldane gas analysis apparatus(9).

In the earlier observations, when one type of blood only was used on one day, the data for construction of the curves could be obtained in about 2½ hours, but in the later experiments, when both arterial and venous bloods were taken at the same time, the whole process occupied about 5 hours. Under these circumstances, it was necessary to be certain that no change, such as by glycolysis, was taking place which might render the later analyses not comparable with the earlier. The first samples analysed were therefore re-examined at the end of the experiment, but no significant changes were found. Further, there was no change in the amount of sugar in the blood.

To make certain that the blood in the tonometers was fully oxygenated, the oxygen saturation of the blood from the tonometer with lowest partial pressure of oxygen was determined in a few instances. In no instance did it fall below 95 p.c. saturation. The Haldane blood gas apparatus(10) was used for estimating the oxygen saturation.

Results. In two experiments, curves were obtained with arterial and with venous blood, and with venous blood drawn with the arm in a hot water bath at 44°C . as described by Meakins(4). The samples were obtained at the same hour on different days. In both subjects there was no reason to expect any change in condition from day to day; one of them (*A*) was a healthy normal, and the other (*B*) was a patient suffering from a chronic nervous condition and was in bed and on a constant diet. The results obtained in subject *A* are given in Table I, and the curves for subject *B* are shown in Fig. 1.

In subject *A* the CO_2 dissociation curve obtained with venous blood

drawn in the ordinary way, without any precaution to avoid stasis, shows a lowered CO₂ capacity compared with the arterial blood; while the points obtained with the venous blood drawn with the arm in hot water lie nearer to the venous curve than to the arterial. The same result was obtained in subject *B*, although the O₂ saturation of the venous blood from the heated arm was practically the same as that of the arterial blood.

A different result was obtained with the pH-CO₂ tension curves, for although the curve obtained with the venous blood from each of the

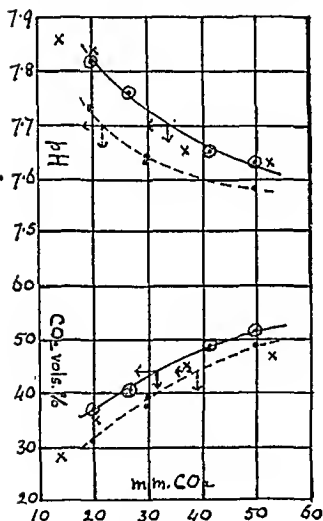


Fig. 1.

Fig. 1. Subject B.

- Arterial blood (30. x. 23). O₂ content = 95 p.c. saturation. O₂ capacity = 17.7 vols. p.c.
- Venous blood: with constriction (27. x. 23). O₂ content = 50 p.c. saturation. O₂ capacity = 18.5 vols. p.c.
- x Venous blood: with arm at 44° C. (12. xi. 23). O₂ content = 94 p.c. saturation. O₂ capacity = 18.4 vols. p.c.

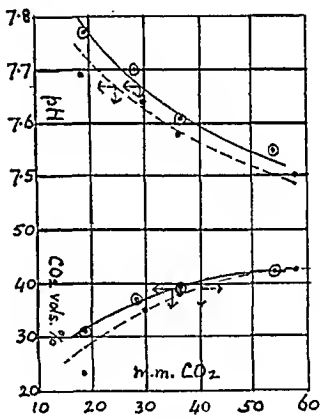


Fig. 2.

Fig. 2. Subject D.

- Arterial blood. O₂ content = 100 p.c. saturation. O₂ capacity = 17.9 vols. p.c.
- Venous blood: without constriction.

The arrows in both figures indicate the CO₂ tension of the arterial blood.

two subjects lies to the acid side of the arterial blood curve, the points obtained with the venous blood from the heated arm fall approximately on the arterial blood curve.

Since the CO_2 capacity of the blood may very well change from day to day, it was decided that the samples of blood to be compared must be drawn at the same time. This, however, necessitated omitting further observations with venous blood from a heated arm, as it was impossible to carry out all the observations in the course of one day. In the later experiments, therefore, comparisons were made only between the curves obtained with arterial blood and with venous blood drawn in the ordinary way, using as little stasis as possible. The subjects utilised in these observations were all patients in hospital wards, and although some of them were convalescents and practically healthy, others were not. Two of the patients were utilised more than once in the course of their stay in hospital.

In subjects *D*, *H*, and *E* (the second observation, 31. iii. 24) the CO_2 dissociation curve obtained with venous blood lies in each case below that obtained with the arterial blood, that is to say, it shows a lower CO_2 capacity. Subject *D* was convalescent from lobar pneumonia, and the venous blood was obtained without any constriction being used. In subjects *H* and *E* constriction was used throughout the withdrawal of the blood, and there was some stasis in each case. Subject *H* was suffering from acute endocarditis, and *E* from heart failure.

In each of these observations the pH-CO_2 tension curve obtained with venous blood lies to the acid side of that obtained with arterial blood, in agreement with the lower CO_2 capacity. The results obtained in subject *E* are shown in Table II, and the curves obtained from subject *D* are shown in Fig. 2.

Three observations were made on subject *F*, who was suffering from heart failure due to arterio-sclerosis, myocardial degeneration and high blood-pressure. At the time of the first observation (11. ii. 24) he had pronounced Cheyne-Stokes respiration and signs of circulatory failure while at rest in bed. The arterial blood was drawn as nearly as possible during a hyperpnoëic phase. At the time of the second observation (25. ii. 24) his condition had improved considerably, but there was still a periodicity in the respiratory rhythm, and at the third observation (17. iii. 24) he was greatly improved and able to be up and about. At each of the three observations the CO_2 dissociation curves with venous and arterial blood coincide, but the pH-CO_2 tension curve obtained with

venous blood on the first occasion is to the acid side of the curve obtained with arterial blood, it is slightly to the alkaline side on the second occasion, while on the third occasion the two curves coincide. On the first occasion no constriction was employed in obtaining the venous blood, while on the second and third occasions slight constriction was necessary to enable the needle to be inserted into the vein, but was relaxed while the blood was being withdrawn. The results obtained on the first occasion are shown in Table II.

In subject *G*, a case of hysterical dyspnoea, the CO₂ dissociation curves coincide, but the pH-CO₂ tension curve obtained with venous blood lies to the alkaline side of that obtained with arterial blood. Constriction of the arm was necessary throughout the withdrawal of the blood from the vein.

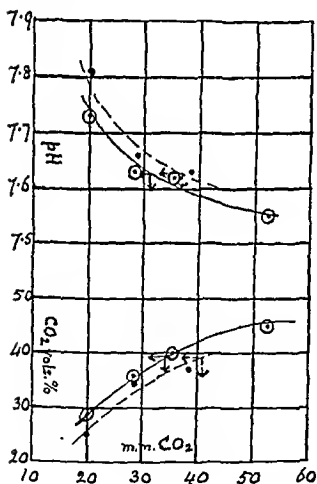


Fig. 3.

Fig. 3. Subject C.

○ Arterial blood. O₂ content = 96 p.c. saturation. O₂ capacity = 16.7 vols. p.c.
● Venous blood.

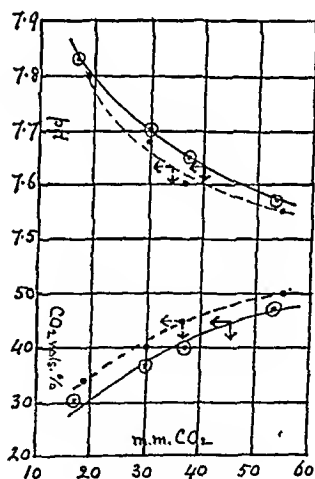


Fig. 4.

Fig. 4. Subject E.

○ Arterial blood (28. i. 24). O₂ content = 87.8 p.c. saturation. O₂ capacity = 17.3 vols. p.c.
● Venous blood.

The arrows in both figures indicate the CO₂ tension of the arterial blood.

In subject *C*, who was convalescent from lobar pneumonia, the CO_2 dissociation curve constructed with venous blood shows a lowered CO_2 capacity compared with the arterial blood curve, but the $p\text{H-CO}_2$ tension curve from venous blood is very slightly to the alkaline side of the arterial blood curve (see Fig. 3). In this case constriction of the arm was necessary to permit the insertion of the needle into the vein, but was relaxed before the blood was withdrawn.

At the first observation on subject *E* (28. i. 24), at a time when she was in a condition of severe heart failure with cyanosis and œdema, the CO_2 dissociation curve constructed from venous blood showed a higher CO_2 capacity than the arterial blood curve, while the $p\text{H-CO}_2$ tension curve from the venous blood lies to the acid side of the arterial blood curve (see Fig. 4). In this case no constriction was necessary, but the condition of the patient and the distended veins showed that considerable stasis was present.

Discussion.

Even omitting the observations done on subjects *A* and *B* on the ground that the samples of blood were not obtained at the same time, and are not strictly comparable, the remaining observations on patients indicate that the CO_2 capacity of venous blood obtained from an arm vein by the usual method is not always the same as that of arterial blood drawn from the femoral artery. It is therefore not justifiable to draw conclusions as to the CO_2 tension in arterial blood by means of CO_2 dissociation curves constructed with venous blood. Haggard and Henderson(8) were the first to indicate that the arterial CO_2 tension could be determined in this way, and Means, Bock and Woodwell(11), Peters and Barr(12), Peters, Barr and Rule(13), Campbell and Poulton(2), Campbell, Hunt and Poulton(1), and others have utilised this method in observations on human subjects. Our results would appear to indicate a possible source of error in their conclusions, and may also afford an explanation of the discrepancies between the calculated arterial CO_2 tension and the CO_2 tension in the alveolar air, noted by Campbell and Poulton, and Peters and Barr.

Dautrebande, Davies and Meakins(4) have shown that stasis causes a lowering of the CO_2 capacity of the venous blood, but they utilised a degree of stasis much greater than that entering into our observations, and we find no lowering of CO_2 capacity in some cases where constriction of the arm was necessary, and in others a definite lowering when no stasis was used. In both subjects in which we examined

the blood taken from an arm immersed in a hot water bath, as recommended by Dautrebande, Davies and Meakins, we find the capacity approximates to that of the venous blood drawn with constriction to the arm and not to that of the arterial blood. A further argument against the view that stasis is the cause of the phenomenon is seen in subject *E*, in whom the capacity of the venous blood from the arm is higher than that of the arterial, in spite of the patient's condition, which indicated the presence of a pathological degree of stasis. It is difficult to believe that the mixed venous blood returning to the heart should have a different CO₂ capacity after it has been oxygenated in the tonometers from that of the arterial blood under the same conditions, and it would appear that the explanation must be sought in the local condition of the arm. Some organs, such as the kidney and stomach, elaborate highly acid secretions; while others, such as the pancreas and salivary glands, elaborate alkaline secretions. When these varied functions of organs are considered, it would seem rational that the capacity for CO₂ of venous blood should vary throughout the body, even though the mixed venous blood in the right heart has the same capacity as arterial blood.

Another possible explanation has been considered, namely, that the rates of glycolysis in venous blood and in arterial blood may be different. Lovatt Evans⁽⁶⁾ pointed out the possible error due to this factor, but we found no loss of blood sugar in the venous blood at the end of the two experiments on subject *E*, and the last experiment on subject *F*.

The fact that the pH-CO₂ tension curve from venous blood may lie to the alkaline side of the arterial blood curve when the CO₂ capacity of the venous blood is less than that of the arterial, or identical with it, and that it may lie to the acid side when the CO₂ capacity is greater than, or identical with, that of the arterial blood, indicates that the pH of blood as determined by the Dale-Evans colorimetric method after dialysis does not depend on the $\frac{H_2CO_3}{NaHCO_3}$ ratio alone. It is not definitely known on what factors the determination of the pH by this method does depend, but Lovatt Evans⁽⁶⁾ concluded that this ratio is one of the factors. Hypothetically, therefore, it should be possible to determine the CO₂ tension of the arterial blood from pH-CO₂ tension curves and the pH of the arterial blood. We find that when venous blood is used for the construction of the curves the figures obtained for the arterial CO₂ tension by this method may differ from those obtained from the CO₂ dissociation curve and the arterial CO₂ volume by as much as 20 mm., but that when arterial blood is used for the construction of the curves

the difference is less than 6 mm. This contrast is seen in the figures obtained in the observations on subjects *A, B, D, E* (second observation), *F* (first observation), *G*, and *H*. In the remaining subjects, *C, E* (first observation), and *F* (second and third observations), the difference is 5 mm. or less, whether venous or arterial blood is used. When the possible sources of error are considered, and especially the errors due to the construction of a curve from three or four points, differences of a few millimetres in an interpolated point may be regarded as within the limits of accuracy of the method. The two methods, therefore, give figures in practical agreement when arterial blood is used, but figures which may differ considerably when venous blood is used, and we consider that this indicates that conditions in the arterial blood cannot be accurately deduced by means of curves constructed with venous blood, but that approximately accurate results can be obtained with curves constructed with arterial blood. The figures, even when arterial blood is used, are not always identical, and that obtained by the $pH-CO_2$ tension curve is as a rule lower than that obtained by the CO_2 dissociation curve; and this discrepancy might be explained if the manipulation to which the blood is subjected, and especially the heating of the blood in the tonometers, caused a slight shifting of the blood to the acid side.

Our observations do not indicate that the differences between the curves obtained by arterial and venous blood depend directly on pathological conditions, such as heart failure, dyspnoea, etc., but more extensive observations would be necessary before it could be stated that disease conditions have no effect in producing the observed differences.

SUMMARY AND CONCLUSIONS.

1. CO_2 dissociation curves and $pH-CO_2$ tension curves constructed with oxygenated venous blood from an arm vein have been compared with curves constructed with arterial blood on eleven occasions in eight human subjects.

2. In a majority of the observations the curves did not coincide. The CO_2 dissociation curves constructed with venous blood and with arterial blood were practically identical in four out of eleven observations. The $pH-CO_2$ tension curves were identical in only one observation out of eleven.

3. The differences observed were not the result of the conditions of stasis under which the venous blood was sometimes obtained.

4. It is concluded that conditions in the arterial blood cannot be accurately deduced by means of curves constructed with venous blood,

but that approximately accurate results may be obtained with curves constructed with arterial blood.

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APPENDIX.

TABLE I. Subject A.

Date	Nature of blood sample	O ₂ p.c. saturation	O ₂ capacity vols. p.c.	CO ₂ vols. p.c.	pH Dale-Evans	Tonometers				Arterial CO ₂ tension	
						CO ₂ partial pressure mm.	Blood		CO ₂ dissociation curve	pH-CO ₂ tension curve	
24. ix. 23	Venous: with constriction	33	23	46	7.53	21.4	30.0	7.58	39	20	
						28.4	34.7	7.55			
						40.8	39.6	7.49			
						48.9	43.7	7.44			
1. x. 23	Venous: at 44° C.	—	19.6	40	7.60	23.6	33.6	7.62	39	27	
						29.6	35.0	7.61			
						36.5	37.5	7.55			
						47.5	43.6	7.51			
8. x. 23	Arterial	93.5	19.9	39.6	7.60	13.0	25.6	7.81	32	27	
						19.1	20.2	7.68			
						29.5	37.8	7.58			
						39.0	42.3	—			

TABLE II.

Subject	Date	Arterial blood				Tonometers				Arterial CO ₂ tension			
		O ₂ p.c. saturation	O ₂ capacity vols. p.c.	CO ₂ vols. p.c.	pH Dale-Evans	Venous blood CO ₂ partial pressure mm.	Blood CO ₂ vols. p.c.	pH	From venous blood curves	From arterial blood curves	CO ₂ dissociation curve	pH-CO ₂ tension curve	pH-CO ₂ tension curve
E	31. iii. 24	99	18.0	44	7.64	18.7 28.1 38.2 58.5	29.2 36.2 42.6 51.0	7.68 7.59 7.55 7.43	13.7 27.0 36.7 52.5	32.5 39.0 43.5 52.0	7.75 7.68 7.60 7.50	40	31
F	11. ii. 24	100	16.8	39.9	7.82	15.9 24.0 34.0 50.5	30.0 39.0 42.7 48.6	7.96 7.81 7.70 7.62	17.3 25.2 37.2 50.0	32.2 37.8 41.0 48.1	7.99 7.83 7.73 7.63	29	27

VASO-MOTOR CENTRES. Part III. Spinal vascular (and other autonomic) reflexes and the effect of strychnine on them. BY J. N. LANGLEY.

(From the Physiological Laboratory, Cambridge.)

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THE aim of the experiments given in this paper was to see if any explanation could be given of the varying results which previous observers have obtained in animals a short time after section of the spinal cord, by stimulating the central end of a sensory nerve both before and after injecting strychnine.

In what follows, I speak, for the sake of brevity, of "stimulating a nerve" instead of "stimulating the central end of a nerve." In all cases the nerve was tied and cut.

Method. The experiments were made on cats. The cats were anæsthetised with chloroform, tracheotomy performed and anæsthesia continued with C.E. mixture till the animal was decerebrated or decapitated. The decerebration was in nearly all cases by the guillotine. Both vagi were cut, the necessary cannulæ inserted, the nerves to be stimulated tied and cut, the spinal cord exposed in the region to be transected. This region was usually the mid-cervical, in two experiments the 8th cervical to 2nd thoracic. A carotid blood-pressure tracing was taken, the spinal cord cut, curari injected in just sufficient quantity to paralyse the somatic motor nerves, the nerves stimulated two or more times. In most cases strychnine was injected in successive doses, and the nerves stimulated two or more times after each injection. This method was used in order to meet the possible objection that in decapitation the

vaso-motor centre is not wholly severed from the spinal cord. By it some idea of the excitability of the spinal centres is gathered from the extent of the rise of blood-pressure produced by the section of the spinal cord. Decapitation¹ was performed after ligature of the arteries by Sherrington's method. In a few experiments, after decapitation, the cord was again cut in the mid-cervical region. Sometimes instead of cutting off the head after section of the cord, the brain was destroyed.

No certain difference was found in the results of nerve stimulation with these and other minor variations of method, *i.e.* the presence of the upper cervical portion of the spinal cord did not appreciably affect the results. The blood-pressure in the individual spinal animals, for the first half hour or more, varied considerably; it was usually 60-70 mm. Hg, sometimes only 40-50 mm., but occasionally more than 100 mm. Hg. The variation was found whichever method of obtaining a spinal animal was employed, but on the average the pressure was lower when the animal was decerebrated, and the cervical cord cut, probably in consequence of the greater loss of blood and the longer duration of dissection. A high blood-pressure probably means stimulation of the cut surface of the cord, either by blood clots or by swabs sometimes used to check oozing from the anterior spinal artery. Ustimovitch(1) found in the dog that after section of the cord, the blood-pressure slowly sank. According to Sherrington(2) the pressure in the cat after decapitation is 70-90 mm., and is higher an hour or so after the decapitation than earlier. In my experiments, in which nerve stimulation was begun soon after cord section, the blood-pressure invariably sank, except for temporary rises when strychnine was injected. The pressure is somewhat greater when the preparation is on its side than on its back owing to slight compression of the liver and other viscera.

Stimulation was by the interrupted current of an induction coil, the current being, as a rule, fairly strongly felt on the tongue. Since it has been shown that the reflex rise of blood-pressure caused by the nerves of the limbs in spinal animals, if it occurs at all, is at most small, the nerve to be stimulated must either be arranged so that it can be stimulated without any movement or pressure on the body, or (preferably) the nerve must be placed on electrodes held in a stand. A large nerve such as the sciatic should be laid between the electrode terminals and not simply laid on them. Further it is not safe to infer an absence of reflex from the sciatic until a very strong current has been used.

¹ After cutting the spinal cord the occipito-atlantoid joint is I think more easily found if the tissues at the side of the neck are first cut up to the vertebrae

A difficulty which not infrequently occurs in the way of determining whether there is or is not a small rise of blood-pressure is that for some time after the cord is cut, there are 3rd order waves¹ in the blood-pressure tracing. These vary greatly in size in different experiments. Usually they are only 2-3 mm. in height but in one experiment waves of 16 to 20 mm. continued for about a quarter of an hour. Whilst they usually occur at fairly regular intervals, they do not always do so, and whether there is a slight reflex effect can only be told by an examination of the whole of the tracing. A complete disappearance of variations in the tracing may mean that the excitability of the spinal cord is very small. It is advisable to test the excitability at the end of an experiment by noting what rise of blood-pressure and erection of hairs is caused by asphyxia.

Observations on un-curarised spinal animals are untrustworthy, since contraction of abdominal muscles or of the diaphragm readily causes a slight rise of blood-pressure. Contraction confined to a leg may do so, but often does not. It must be borne in mind that curari paralyses the muscles of the diaphragm later than those of the limbs and trunk.

SPINAL VASCULAR REFLEXES FROM LIMB AND TRUNK NERVES.

Reflexes on carotid² blood-pressure.

Limb nerves. In the early experiments in Ludwig's laboratory, no rise of general blood-pressure was obtained in rabbits by stimulating the sciatic after section of the spinal cord just below the spinal bulb. An absence of result has also been obtained by several subsequent observers, but some have found that a rise or fall of carotid blood-pressure of a few millimetres of mercury was at times, though not constantly, obtained. In rabbits and cats an effect, when obtained, was a rise of blood-pressure, in dogs it was usually a fall.

Owsjannikoff(3) obtained in the rabbit no rise of blood-pressure from the sciatic, S. Mayer(4) found none in the dog, Dittmar(5) confirmed Owsjannikoff's result in the rabbit. Schlesinger(6) obtained none from the median nerve Bochefontaine(7) tied the spinal cord of the dog above the axis and found that the sciatic caused a rise of blood-pressure of 20-25 mm Hg Whether this was a spinal reflex is doubtful since after the ligature, section of the dura mater of the brain caused slowing of the heart and a still greater rise of blood-pressure Kabierski and Heidenbain(8) in experiments on rabbits in which the carotid and vertebral arteries were tied obtained a rise of blood-pressure from the sciatic in 7 out of 20 experiments, the rise varying from 1-10 mm. Hg. When the spinal cord was cut, a rise was more rarely obtained Dogs were said to be less, and cats

¹ I include in these all waves which cannot be considered as synchronous with variations in the size of the thorax.

² The blood-pressure as taken in the carotid is of course the aortic blood pressure.

more favourable for obtaining a trifling rise. Luchsinger(9) seldom found a rise from the sciatic in cats and rabbits and when it occurred it was trifling. Stricker(10) sometimes obtained a rise up to 50 mm. Hg from the sciatic in dogs, but as curari was not given it is uncertain how far the rise was due to muscular contraction and movement, and apparently he found no rise after curari had been injected. Smirnoff(11) found that when the spinal cord was cut in the dog just above the 1st thoracic vertebra the brachial nerves had no effect, and the sciatic caused either none, or a slight rise with the first stimulus only; that when the cord was cut below the 6th vertebra the sciatic nerve had no effect and the brachial nerves also had none, or caused a slight fall with the first stimulus only, but that after section between the 1st and 6th thoracic vertebræ, the brachial nerves caused a fall of blood-pressure and the sciatic caused a rise. The negative results in these experiments are undoubtedly untrustworthy. Ustimovitch(1) usually obtained from the sciatic of the rabbit a rise of 2 to 4 mm., the maximum being 8 mm.; in the dog the sciatic frequently caused a fall of blood-pressure and very seldom a rise. Muscular movement was not prevented by curari. Thayer and Pal(12) found that both the sciatic and brachial nerves in the dog caused a fall of blood-pressure and caused it after section of the splanchnic and of the limb nerves; the sciatic still caused a fall after section of the cord in the thoracic or lumbar region. Asher and Lüscher(13) obtained a very small reflex rise from the sciatic in rabbits made spinal by injecting paraffin into the carotid arteries and un-curarised. Jappelli(14) stimulated the sciatic in the dog with a series of brief tetanic currents during cessation of artificial respiration. In curarised animals, he found no effect at first but somewhat later and before any marked asphyctic rise of blood-pressure, he obtained a series of rather irregular slight rises which tended to be synchronous with the stimuli. When curari was not given, a slight rise was caused by each of a series of single induction shocks. Pike(15) obtained no effect from the sciatic of the cat after giving curari. Sherrington (16), p. 143) very rarely obtained in cats any vaso-motor reflex from the sciatic and/or other somatic nerve.

The sciatic or one of its branches—usually the musculo-cutaneous (superficial peroneal) was stimulated after section of the spinal cord and injection of curari in 18 experiments besides those to be mentioned later in which the abdominal viscera were exposed or excised. In 15 of these experiments the nerve was stimulated (1 to 5 times) in the first half hour after cord section and strychnine was then injected. In the last three (cp. p. 238) the stimulations were for a longer period before strychnine was injected.

In nine experiments there was a definite though trifling rise of blood-pressure. The rise varied with the successive stimulations usually from $1\frac{1}{2}$ to 5 mm. and in one experiment from $1\frac{1}{2}$ to 7 mm. When shallow 3rd order waves were already present the stimulation increased the height of one or two of them. When the curve ran an even course, stimulation usually caused two waves of rise of pressure and sometimes more. Fig. 1 gives two typical examples of the effect of sciatic stimulation.

In six experiments there was a rise of pressure of 1 to 2 mm. with one or two of the stimulations, but in an equal or greater number of stimulations there was no effect.

In three experiments, stimulation had no effect, in two of these, however, the nerve was stimulated once only before giving strychnine

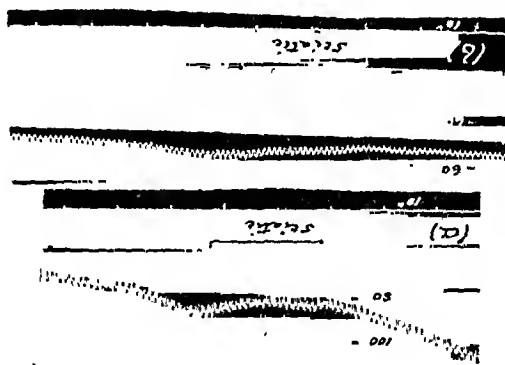


Fig. 1. Cat, decerebrated, vagi cut, curarised. (a) Spinal cord cut between 3rd and 4th cervical segment. Stimulation of sciatic near the end of the rise of blood-pressure caused by the section. (b) From another experiment. Cord cut between 1st and 2nd thoracic. Stimulation when the blood-pressure had fallen to a nearly constant level.

(cp. Exp. 1, p. 243). There were two other experiments in which the sciatic had no effect, but in these the carotid pressure was only 30–25 mm. Hg and section of the cord caused a small rise only, showing that the excitability of the cord had greatly decreased.

In eight experiments, one or more of the brachial nerves was stimulated. The results were much as those with the sciatic but on the whole the rise was less (though once it was 7 mm.) and was less frequently obtained.

In connexion with observations on the degree of restriction in the viscera of the vascular reflexes (p. 240) four experiments were made in which the spinal animal was placed in Ringer's fluid at 38–39° C., the abdominal viscera exposed and the fore and hind leg nerves stimulated. In each of these a slight rise of blood-pressure was obtained. The rise from the median, ulnar and superficial peroneal was 2–4 mm. The sciatic was stimulated in one experiment only, it caused a rise of 7–8 mm. and it set up a series of 3rd order waves (Fig. 2).

Lower thoracic and upper lumbar cutaneous nerves. It seemed possible

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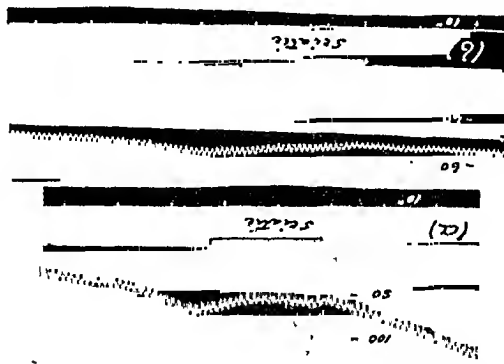


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(cp. Exp. 1, p. 243). There were two other experiments in which the sciatic had no effect, but in these the carotid pressure was only 30–25 mm. Hg and section of the cord caused a small rise only, showing that the excitability of the cord had greatly decreased.

In eight experiments, one or more of the brachial nerves was stimulated. The results were much as those with the sciatic but on the whole the rise was less (though once it was 7 mm.) and was less frequently obtained.

In connexion with observations on the degree of restriction in the viscera of the vascular reflexes (p. 240) four experiments were made in which the spinal animal was placed in Ringer's fluid at 38–39° C., the abdominal viscera exposed and the fore and hind leg nerves stimulated. In each of these a slight rise of blood-pressure was obtained. The rise from the median, ulnar and superficial peroneal was 2–4 mm. The sciatic was stimulated in one experiment only, it caused a rise of 7–8 mm. and it set up a series of 3rd order waves (Fig. 2).

Lower thoracic and upper lumbar cutaneous nerves. It seemed possible

that the lower thoracic and lumbar nerves, since they arise from the region of the spinal cord containing the vaso-constrictor nerve cells,



Fig. 2. Spinal cat (1st cerv.; brain destroyed, curarised) in warm Ringer's fluid, abdominal viscera exposed. Two stimulations of the sciatic.

might have a greater effect than the sciatic. Nine experiments were made in which the cutaneous branches of one or more of the 13 thoracic to 2nd lumbar nerves were cut and the central ends stimulated. In two experiments there was no effect, in one it was doubtful whether there was an effect or not, in two there was a trace of rise of blood-pressure, in two the rise varied from $\frac{1}{2}$ to 1 mm. Hg; and in two the rise varied from 2-7 mm. Hg. The results then were much the same as those obtained with the limb nerves. Though the number of nerve fibres stimulated was less.

The foregoing experiments show that a trifling rise of general arterial blood-pressure can usually be obtained from the limb and trunk nerves of the spinal cat. The fact suggests that an absence of reflex rise must be due to some experimental condition. A considerable loss of blood, a low blood-pressure, a longer duration of low blood-pressure are unfavourable for the production of a reflex rise, but not a few of the variations in the results could not satisfactorily be explained by variations in these conditions. Loss of blood in somewhat greater quantity than usual seemed to make the results inconstant, but increasing its volume—and the blood-pressure—by injecting gum saline did not make the results more constant. Raising the blood-pressure by adrenaline, or by stimulation of the spinal cord had also no constant effect on the

reflexes. Low blood-pressure, if not too prolonged, did not necessarily abolish the reflex; thus in one experiment the blood-pressure fell to 27-24 mm. Hg, yet the sciatic caused a rise of $1\frac{1}{2}$ mm. in each of three stimulations in the subsequent quarter of an hour. In this experiment the mid-cervical region of the spinal cord was frozen with liquid air before being cut; since its section caused no rise of blood-pressure, the occurrence of a reflex rise might be attributed to an absence of fatigue, but sometimes the sciatic will cause a rise of blood-pressure almost immediately after a very large rise has been caused by stimulation of the cord either mechanically by section (cp. Fig. 1, *a*) or by electrical stimulation. Moreover such variations in condition do not account for the variable effect of successive stimulations, in any one experiment. For whilst the effect depends to some extent on the interval between the stimulations, the effect may also be variable when the interval is two to three minutes. Section of the cord no doubt produces different degrees of "shock," i.e. of depression of activity of the spinal centres in different cats, but judging the excitability by the response to electrical stimulation, strychnine and asphyxia, the spinal vaso-motor centres may be very excitable and yet fail to give a reflex to one or more stimulations of a limb or trunk nerve.

These considerations suggested that one of the factors causing variation in the reflex rise, in addition to those mentioned, is a difference in the response of the peripheral vessels. If such existed, it seemed probable that it would be in the vessels of the abdominal viscera, and as a first step in the investigation of this, the effect of removing the abdominal viscera was tried.

Reflex on carotid pressure after evisceration. In four experiments, the intestine, stomach, pancreas, and spleen were excised, and in one of these the left kidney and adrenal gland also. If, in doing this, the intestine is exposed, it contracts, nearly empties its blood vessels, and the blood-pressure is for a time increased; in one case, the carotid pressure, even after injecting curari was 118 mm. Hg. In one or other of the experiments, the posterior tibial, the superficial peroneal, the sciatic, and the median nerve were stimulated. Most stimuli had either no effect or a doubtful one. With none was there a rise of more than 2 mm. Hg. The experiments indicated that the reflex rise ordinarily obtained is chiefly due to contraction of the vessels of the viscera, but that the contraction is not confined to these.

Effect of digestion. In 15 of the 18 experiments mentioned above, the animal was fed the evening before being anaesthetised and the remains

of the meal may have been eaten in the morning, *i.e.* the animals may have been in different digestive states. In order to determine whether the state of digestion influenced the reflex rise, the last three experiments were made on cats fed with milk, or milk and meat, two hours before being anæsthetised. In each, a slight rise of blood-pressure was obtained by stimulating the sciatic. In two of the experiments a closely similar rise was obtained with each of more than half a dozen stimulations; one of these is shown in Fig. 3. In the third experiment the rise was variable and inconstant. But I think it may be concluded that one of the chief factors determining the variability in the occurrence of a reflex rise of blood-pressure is the state of digestion. As will be described later, digestion has a great influence on the reflex rise of pressure after strychnine

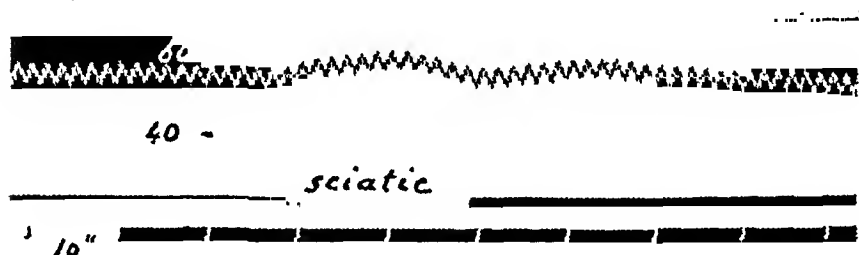


Fig. 3. Cat in full digestion. Cord cut at 1st cervical segment, brain destroyed, curarised. The figure shows one of a series of eight sciatic stimulations, each giving the same effect.

has been injected. The more constant effect in digestion is probably due to an increase in the volume of the blood, though, as I have said, I have not found that injecting gum saline has any certain effect in increasing the reflex rise of blood-pressure or in making it more constant.

In view of the results in the digesting spinal animal and of the frequency with which a trifling rise of blood-pressure is obtained in hungry or slightly digesting spinal animals, I conclude that when afferent nerve stimulation causes no rise of blood-pressure it is in consequence of one or more of the experimental conditions which tend to prevent it, notwithstanding that in some cases the conditions concerned are not definitely ascertainable.

Local reflexes and reflexes other than vaso-constrictor.

The small rise of general arterial pressure caused by the limb nerves in "acute" experiments on spinal cats can hardly be regarded as of any

importance in modifying the circulation in normal conditions. So far there is little reason to doubt the theory that spinal vaso-constrictor reflexes are normally unimportant¹. But this is only true if the reflex vaso-constriction is widespread. At a certain degree of limitation of the area of vaso-constriction, the reflex would become important, for the smaller the area, the more completely the blood must be cut off from it in order to cause a rise of a few millimetres of general arterial pressure.

That the spinal cord serves the purpose of producing local constriction of blood vessels has been suggested by Vulpian⁽¹⁷⁾ and by Kabierski and Heidenhain⁽⁸⁾ and by some later observers. Neither the experiments of Vulpian nor of Kabierski and Heidenhain are satisfactory on this point. Vulpian on stimulating the sciatic (dog) on one side after section of the spinal cord found a fall of temperature in the foot of the opposite side. How much of the rest of the body was affected was not determined. The local action was presumably deduced from his observation that stimulation of the toes or web caused a rise of temperature in the foot on the same side. No evidence, however, was given that the effect was produced reflexly, for local dilatation is readily produced by direct stimulation. Kabierski and Heidenhain stated that no reflex rise of blood-pressure was produced from the sciatic after section of the cord in the lower thoracic region and argued that this was a consequence of the vaso-constrictor effect from the upper part of the cord being cut off so that the area affected was too restricted to cause a rise. The statement is inconsistent with their suggestion of local action. At this date the extended area innervated by the sympathetic fibres of each spinal nerve was imperfectly appreciated. With our present knowledge it is practically certain that no spinal sympathetic reflex can be confined to the area of a single spinal nerve. Except in cases of exaggerated local excitability the minimal area is probably that of three nerves. To determine the area affected in the skin would require numerous experiments by the thermo-electric method, but ocular inspection affords a simple means of determining whether any considerable local vaso-constriction occurs.

Comparative observations were made on the colour of the fore and hind feet and on the colour and contraction of the viscera in cats after decapitation or destruction of the brain.

Colour of feet. In different experiments, the posterior tibial nerve, the

¹ This argument would of course not hold if it could be shown that section of the cord decreases the excitability of a part of the reflex arc with which the descending spinal nerve fibres are not connected.

superficial peroneal nerve on one or both sides and the sciatic were stimulated. In the fore feet no certain change of colour was seen. In the hind feet there was sometimes, but not constantly, slight paling of both hind feet, or slight flushing, or slight paling preceded by flushing; the flushing was more frequently on the side opposite to that stimulated. Thus there was evidence of a trifling vascular reflex, both vaso-constrictor and vaso-dilator, not extending to the fore feet, but it was clear that no marked vaso-constrictor or vaso-dilator reflex occurred. The blood-pressure in these experiments was 50-65 mm. Hg; the flushing would no doubt have been greater with a normal blood-pressure.

Change of colour and other effects on the viscera. In four experiments, after section of the spinal cord at the 1st cervical segment, decapitation or destruction of the brain and curarisation, the spinal animal was placed in Ringer's fluid at 38-39° C. and the abdominal viscera exposed. The superficial peroneal nerve and one or more of the nerves of the fore limb above the elbow were stimulated. The effect of successive stimulations was not constant, but sometimes the hind limb nerve caused distinct pallor of the proximal colon, and apparently some of the lower part of the ileum, whilst the fore limb nerves caused pallor of the duodenum and apparently of the stomach. After several stimulations the middle part of the intestine was markedly less pale than the rest.

Incidental observations were made on other reflexes. The hind limb nerves caused slight but distinct contraction of the bladder and this was not seen on stimulating the fore limb nerves. It may be recalled that Schlesinger⁽⁶⁾ in spinal rabbits obtained contraction of the uterus on stimulating the sciatic nerve, sometimes by stimulating the anterior crural but not by stimulating the brachial nerves.

In each experiment anti-peristaltic (anastaltic) waves occurred in the proximal colon. Stimulation of the superficial peroneal nerve appeared to increase these, and occasionally to stop them; occasionally, too, its stimulation was followed by inhibition of the rhythmic movement of the ileum, suggesting a fairly general slight action on the autonomic centres of the lower part of the spinal cord.

The results show I think some degree of local reflex action from the limb nerves, but they do not show any great restriction of effect. I had hoped that the degree of local action could be definitely determined by observing the areas in which erection of the hairs occurred. Unfortunately for this aim, stimulation of afferent nerves in the spinal animal does not cause in the ordinary conditions of experiment, any trace of hair erection. In the numerous experiments on the limb nerves,

no movement of the hairs was seen either before or after injecting strychnine, nor did the splanchnic nerve cause erection unless the electrodes were placed on it dangerously near the sympathetic trunk. Nevertheless it is not to be concluded that afferent nerves are in all conditions incapable of acting on the spinal pilo-motor centres. In three experiments stimulation of an upper lumbar nerve just under the edge of the longissimus dorsi musculo caused erection of hairs, twice confined to the lower lumbar and sacral region, and once to this and the tail region. In each experiment the pilo-motor effect was obtained at the end of an experiment and the blood-pressure was only about 30 mm. Hg when the cord was no doubt in a semi-asphyctic condition. In some cases I have found that stimulation of a lumbar or limb nerve in the stage of asphyxia in which the hairs are beginning to become erect will cause an increase of rate of erection in the hairs of the sacral and tail regions. On the whole I think that the pilo-motor reflex when obtained is due to a summation of asphyctic and afferent nerve stimulation. In any case, the reflex is useless for determining the degree of local action in the ordinary condition of a spinal cat.

EFFECT OF STRYCHNINE ON SPINAL REFLEXES FROM THE LIMB NERVES.

Carotid blood-pressure.

The effect of strychnine¹ on reflex changes of blood-pressure in spinal animals has not been investigated by many observers. The most complete observations are the early ones of Schlesinger⁽²⁾. Schlesinger found in the rabbit that whilst after section of the spinal cord, stimulation of the median nerve had no effect, a more or less large rise was obtained in 18 out of 31 experiments by stimulating the nerve after injection of strychnine. Kabierski and Heidenhain⁽³⁾ mention incidentally that after strychnine has been given to a spinal rabbit, stimulation of the sciatic may cause a large rise of blood-pressure. In similar experiments Asher and Luscher⁽¹³⁾ found some, but only a small rise of pressure in the spinal rabbit. Pike⁽¹⁵⁾ found no rise in the spinal cat on stimulating the sciatic after any dose of strychnine.

In the previous accounts of the action of strychnine in Parts I and II (18, 19) I have given instances in which the sciatic, after injection of a small amount of strychnine, caused a rise of blood-pressure (cp. Exp. 8, Part I and Exp. 2, Part II). In all the subsequent experiments made on spinal cats, a rise of blood-pressure was obtained by stimulating a hind

¹ Strychnine is used for strychnine nitrate.

limb nerve after strychnine had been injected, except in two or three in which the excitability of the cord—as shown by direct stimulation, or injecting strychnine—was greatly decreased.

The general effect of strychnine on the reflex rise of blood-pressure depends within narrow limits on the amount given. But the extent of the rise of blood-pressure in each stage varies widely in different cats. The variations are no doubt in part due to variations in amount of blood lost, duration of dissection, vigour of the animal and so forth. But the chief conditions which influence the extent of the rise of blood-pressure is I think hunger and digestion. As I have said, nearly all my experiments were made in the morning on animals fed the night before (p. 238), and that three experiments were made on animals fed two hours before they were anæsthetised. In the fed animals, the reflex rise of blood-pressure after strychnine was much greater and more constant than in the earlier experiments. It will be convenient to defer an account of the results in animals in full digestion, and give first those obtained in the much larger number of experiments on those in hunger and slight digestion.

(a) After a certain small amount of strychnine ($\cdot 1$ to $\cdot 2$ mg.) which either is just sufficient to cause a slight rise of blood-pressure, or which is just insufficient to cause one, stimulation of a hind limb nerve will produce a greater rise than before, commonly one of 20 to 40 mm. Hg. On successive stimulation the rise decreases, but on injecting the same small amount the rise is again greater, and again falls with successive stimuli. The rate of decrease of effect varies. It depends partly on fatigue for it is faster if the stimuli follow one another quickly, and there may be some recovery if after two stimulations at an interval of half a minute, an interval of two minutes is left. It is partly due to decreased concentration of strychnine for a decrease in rise of blood-pressure still occurs if an interval of several minutes is left between the first and second stimulation, and it is less than after other injections if the first stimulation is delayed for several minutes. The extract given below (Exp. 1) is from one of the experiments in which the sciatic had no effect before strychnine was given, and in which there was an unusually rapid decrease in effect after a small dose of strychnine.

In the less numerous experiments on the effect of minimal doses of strychnine on the lower thoracic, upper lumbar and brachial nerves, similar results were obtained, but the increase in the rise of blood-pressure was less.

(b) After an amount of strychnine which produces an approximately

Exp 1. Cat. Decerebrated and curarised.

Time in mins	Blood-pressure mm. Hg		Rise of blood pressure on stim. sciatic
0	43	Spinal cord cut at 3rd cervical, rise of 49 mm	
		Tie and cut sciatic	0
		Stim sciatic. Sec coil at 10 cm.	0
4	39	Inject 0.5 mg strychnine* (jugular)	
	40	Stim sciatic	Trifling waves
9	40	0.5 mg. strychnine* (slight waves)	
	42	Three stim. of sciatic at intervals of about a minute	23, 0, 0
16	44	0.5 mg strychnine*	
		Sciatic as before	33, 3, 4
		Interval 3 mins—sciatic—sec. coil at 9 cm	20, 6
28	41	0.5 mg strychnine*	
	43	Stim sciatic	32
		Later there were spontaneous rises of blood pressure	
		Four injections of strychnine totalling 2.3 mg were made	
60	52	Stim lower end of severed spinal end for 10 secs—a rapid rise of 108 mm Hg	

* The blood pressure curve remained smooth. There was a trifling slow rise of 1 to 2 mm only.

maximal rise of blood-pressure (2 to 3 mg. sometimes less) most stimulations of the hind limb nerves cause a rise of blood-pressure of 4 to 8 mm. of Hg, whether or no an effect has been obtained before injecting strychnine, but some of the stimulations are usually ineffective. Subsequent injections of 2 to 5 mg. gradually reduce and finally abolish the reflex rise. Each injection is apt to cause brief paralysis. Since the injection of the larger amount of strychnine commonly sets up 3rd order waves, the effect of stimulation can often only be deduced with certainty by an examination of the whole tracing. A similar rise may be obtained by pinching the foot or tail, but I very rarely found any effect from tactile stimuli, viz rubbing the hairs backwards and forwards.

Occasionally when the larger amount is injected, the effect is slight at first and gradually increases. Thus in one experiment the dorsal nerve



Fig. 4 Spinal curarised cat (3rd to 4th cerv) Stimulation of the dorsal nerve of the foot 20 mins after injection of 2 mg of strychnine.

relatively small height, occurred however later in the experiment after (evisceration and) clamping the adrenal veins. The first stimulation in

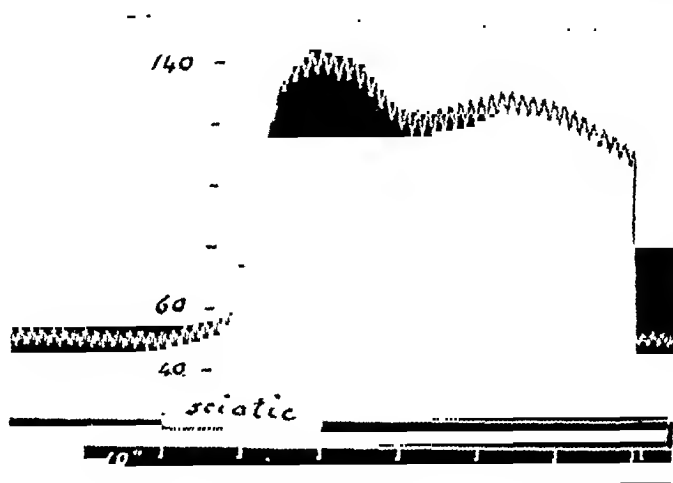


Fig. 6.

the other experiments gave curves of the same form, but the 2nd rise lessened with subsequent stimulations and in one disappeared (Fig. 7).

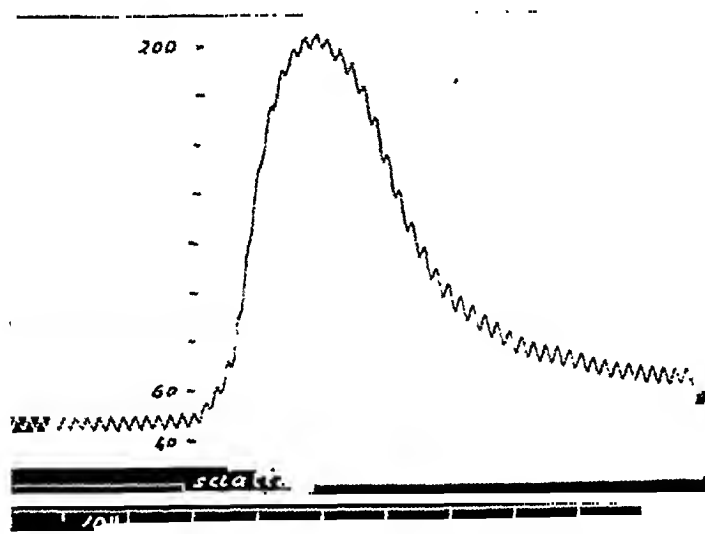


Fig. 7. Spinal cat (3-4 cerv.), curarised. The strychnine injected in this experiment was about that which usually itself causes large rises of blood-pressure, but there was no large rise except when the sciatic was stimulated. The figure shows the effect of the 5th stimulation when there was no secondary rise in the curve.

How far a discharge of adrenalin influences the form of the curve I have not determined. It depends no doubt, as in the intact animal, on the state of the adrenal glands. In animals with intact nervous system it has been shown by Stewart and Rogoff (20) that strychnine causes liberation of adrenalin, but a maximal rise of blood-pressure can be caused by strychnine after removal of the adrenals (17, p. 157). It is not probable that liberation of adrenalin can be produced reflexly as a primary effect.

I have said (p. 243) that in hungry and slightly digesting spinal animals a considerable rise of blood-pressure may occur in recovering from a relatively large dose of strychnine. This was also found in one of the digesting animals. Five mg. of strychnine were injected (after 1.5 mg. in small doses). The rises of blood-pressure caused by successive stimulations of the sciatic in the following 20 mins. (during which the pressure sank from 109 to 78) were: 9, 13, 16, 30, 76.

From the results given above the following conclusions may be drawn. Strychnine in increasing concentration progressively increases the excitability of the spinal centres, so that the reflex rise increases until there is a spontaneous discharge of nerve impulses, and when this has occurred the reflex excitability greatly decreases and is gradually abolished with further increase of strychnine concentration. The several nerve cells are not simultaneously affected except by the larger amounts of strychnine and the successive rises of blood-pressure which occur are not, or only to a relatively small extent, due to discharges from the same group of cells, but to discharges from different cells. On these lines the varying result of afferent nerve stimulation found by others and by myself are intelligible, for the extent of the reflex effect depends on the exact stage of strychnine action at which the nerve is stimulated. Since in the non-digesting spinal animal after about 2 mgm. of strychnine the limb nerves cause a trivial rise of blood-pressure only, it is to be expected that sometimes, in consequence of a decrease of excitability of the spinal cord, not always avoidable by the methods used, no reflex rise would be obtained. The occasional gradual increase in reflex rise after 2 to 5 mg. of strychnine I take to be due to a decrease in the concentration of strychnine in the cells combined with a circulation sufficiently rapid to prevent the fall in spinal excitability which commonly occurs.

Relative effects of strychnine on visceral and cutaneous vascular reflexes.

Effects seen on injecting strychnine. In the spinal cat the injection of strychnine in an amount which causes a marked rise of blood-pressure causes usually a primary brief flush of the feet, followed by pallor. The flushing though distinct is not very great, it usually precedes the

rise of blood-pressure and gives way to pallor soon after the rise of pressure begins. At the height of the pressure rise—if this is considerable—the feet become dead white, even the small veins of the toes disappearing from view. As the blood-pressure falls, there is more or less return of the tint. In the experiments, several injections of strychnine were given beginning with a dose causing little or no rise of blood-pressure, and the maximum total dose was 5 mg. Probably the flushing would have been greater if the larger dose had been given primarily.

Observations were also made on the abdominal viscera exposed in warm Ringer's fluid. In the early period of exposure no distinct primary flush of the intestine was seen but there was distinct pallor when the blood-pressure rose; it was much less than that found (in other experiments) in the feet. In the later stages after exposure of the viscera (after about half an hour) in the warm bath, the pallor of the intestine accompanying a rise of blood-pressure lessens, and it may remain distinctly flushed during large rises of pressure caused by strychnine. In one experiment indeed the intestine remained red and seemed to become rather more flushed during a rise of blood-pressure from 80 to 180 mm. Hg.

Reflex changes in the colour of the feet. After a small amount of strychnine has been injected (·1 to 2 mg.) the sciatic (and any one of its main branches) causes easily observable changes in the colour of the feet. Whenever the sciatic causes a marked rise of blood-pressure, all the feet become pale, and if the rise is large, they become a dead white. The time of beginning of pallor, however, varies. There is commonly some flushing as a primary effect. In some cases the flushing is very trifling, and is not caused by every stimulation; this was so in two experiments on eviscerated animals, and the flushing was only slight in two experiments on hungry non-eviscerated animals; usually the pallor began with the rise of blood-pressure, continued during more or less of the fall of pressure, gradually returning to the previous state. In the three experiments on animals in full digestion primary flushing was marked and constant, its duration varied. Sometimes it began a little before the rise of blood-pressure, increased during the rise and began to decrease as the pressure began to fall. Sometimes it did not begin to decrease till the pressure was half way to its original level. Similar variations occur when the strychnine (in small amount) causes "spontaneous" rises of blood-pressure.

Since the vascular changes on sciatic stimulation occur in the fore feet as well as in the hind feet, the afferent nerves of the limb must be able to set in action the whole range of spinal vaso-motor nerve cells.

There is however evidence of a greater effect on the more posterior nerve cells. In the hind foot, on the opposite side to that on which the sciatic is stimulated, distinct though slight, flushing may occur without any change in the fore feet, and marked flushing when produced, occurs earlier and lasts longer in the opposite hind foot than in the fore feet.

A striking fact is that the vascular changes in the foot on the side on which the sciatic is cut are of the same nature as those on the side with sciatic intact and only differ from them in intensity and duration. Not only so, but they are of the same nature when the anterior crural nerve is cut at Poupart's ligament, the sciatic tied and cut at the sciatic notch and the central end of the sciatic stimulated. The common result of the stimulation is that all the feet flush, the contralateral hind foot most; when the blood-pressure has fallen a little, the fore feet begin to become pale; a few seconds later the homolateral hind foot begins to get pale, and a few seconds later still, the contralateral foot. The occurrence of flushing on the side with limb nerves cut indicates that it is a passive effect caused by constriction elsewhere. The greater flushing on the contralateral side indicates an active effect from vaso-dilator fibres. The eventual pallor on the homolateral side indicates contraction of the abdominal aorta or external iliac artery. But there are other possibilities and these I am investigating. I may mention that I have found essentially similar changes after evisceration combined with clamping of both adrenal veins.

Reflex changes in the viscera. The effect of strychnine on the abdominal viscera was not very striking. Any considerable reflex rise of blood-pressure caused pallor of the whole intestine, but when there was no considerable rise, the action of the sciatic appeared to be on the lower part of the intestine and on the pelvic viscera, and the action of the brachial to be on the stomach and duodenum, i.e. there was simply a slight increase of the effects obtained before strychnine was given. The anastaltic waves in the proximal colon increased greatly during the experiments, but how far this was due to exposure in warm Ringer's fluid and to a direct action of strychnine was not determined. It has been mentioned that Schlesinger⁽⁶⁾ found that the brachial nerves had no effect on the rabbit's uterus after section of the spinal cord. He found, however, that when strychnine had been given, the brachial nerves caused strong uterine contraction.

VASCULAR REFLEXES FROM THE SPLANCHNIC NERVE AND THE
EFFECT OF STRYCHNINE ON THEM.

Observations on the effect of the splanchnic nerve in the spinal animal appear only to have been made by Asher and Lüscher⁽¹³⁾ and by Sherrington⁽¹⁶⁾. In the rabbit Asher and Lüscher very rarely obtained any effect. In the cat Sherrington found that the splanchnic constantly caused a rise of blood-pressure. In his "Mammalian Practical Exercises"⁽¹⁶⁾ a tracing (taken under his direction by a student) is given showing a rise of 9 mm. Hg, but he informs me that he has commonly found the rise to be greater than this—up to 25 mm. Hg.

In a few experiments made in 1919 I found, as had been found by Sherrington, that stimulation of the central end of one splanchnic nerve caused in each experiment a rise of blood-pressure (up to about 16 mm. Hg) and further that after 1–2 mg. of strychnine had been injected, the rise was increased to a variable extent (up to about 30 mm. Hg). When Dr Uyeno was working in the Laboratory in 1923, I asked him to repeat the experiments with a view of determining the constancy of the results. He made a number of experiments and from these most of the details given below are taken. The splanchnic nerve was exposed by dorsal dissection. Since the nerve by this method is isolated nearly up to its point of separation from the sympathetic trunk, care must be taken when stimulating it, to avoid spread of current to the trunk. Spread of current is shown by erection of hairs in the lumbar or in the lumbar and sacral regions. In most of the experiments the cat was decapitated; in five, the stomach, intestines, spleen and pancreas were excised. In three experiments after decapitation, the cord was again cut at the 3–4 cervical segment. The nerve was stimulated for about 20 seconds, two to four times before injecting strychnine, and a variable number of times after each injection. It may be mentioned that none of these experiments were made on cats in full digestion.

In the non-eviscerated cats, the splanchnic nerve gave a rise of blood-pressure with each stimulation. In 8 out of 15 experiments the rise was much the same, and was fairly constant with the successive stimulations, *e.g.* (a) 7, 6, 5.5 mm., (b) 7, 4, 6 mm., (c) 10, 8 mm. In four experiments it varied from 14 to 24, *e.g.* (a) 22, 18, 14 mm., (b) 14, 14, 22, 24, 16 mm. The highest rise obtained was 33 mm. The peripheral end of the splanchnic was for comparison stimulated in a few cases; it always caused a greater rise than that caused by stimulating the central end.

In 12 experiments the effect of the injection of strychnine (·5 to

2 mgm.) was to double approximately the splanchnic rise, but there was considerable variation in detail. (i) In most cases the rise became less with the successive stimulations; examples of this in different experiments are: (a) 36, 16, 10, (b) 22, 15, (c) 42, 12, 10, 8 mm. Hg. The rate of decrease depended partly on the interval allowed between the stimulation and was less if the interval were 2-3 minutes than if it were 45-60 seconds. (ii) In some cases, this progressive decrease did not occur after one or more of the early injections. Thus in one experiment after the second injection of 1 mg. strychnine, successive stimulations caused rises of 24, 18, 20, 26, 24, 20 mm. Hg. The difference no doubt depends mainly upon the rate of decrease of strychnine concentration in the blood. (iii) In most cases, each injection up to a total of 6 mgm.—the maximum given—caused again an increase in splanchnic effect, but in some cases increase was only obtained after the first, or after the first and second injection, the subsequent ones being followed either by no increase or occasionally by an absence of effect. The result was probably due less to a paralysing action of strychnine than to a decrease of excitability of the spinal cord in consequence of continued deficient circulation. (iv) In three experiments, in two of which the cord excitability was certainly decreased, the effect of strychnine was trivial, and there was only a slight and inconstant increase of the effect of splanchnic stimulation.

The results closely correspond to those of stage (b) of the action of strychnine on the effect of sciatic stimulation (cp. p. 242). After .5 or 1 mg. one might expect the spontaneous contraction to be sometimes sub-maximal and that the splanchnic would then give a large rise. A large rise (60-70 mm.) was twice obtained, but there can be little doubt that with smaller amounts of strychnine the three stages present on stimulations the sciatic after strychnine would also be found on splanchnic nerve stimulation.

In the five eviscerated spinal cats, the reflex rise was on the whole less and more variable. The rises with successive stimuli were (a) 6, 8, 2, (b) 8, 4, 2, (c) 8, 4, 6, (d) 3, 0, (e) 2, 0, 0, 2, 6. It is, however, clear that the splanchnic nerve can cause vaso-constriction in the spinal cat in some area other than that of the abdominal viscera. The effects of strychnine on the reflex rise of blood-pressure were similar to those in non-eviscerated animals, but in two only of the experiments was the rise considerable (up to 40 mm.), and the effect of successive stimuli varied widely. In one experiment in which the blood-pressure fell to 40 mm. Hg, the increase in effect after strychnine, though fairly constant, was small. In the experiment mentioned above in which the rises of

cord a little below the section than in the more peripheral part. Head and Riddoch found that reflex sweating was much more profuse in the part of the body receiving sympathetic fibres from the upper region of the severed cord than in that receiving such fibres from the more peripheral region. The most probable cause of the increase of reflexes is I think that the degeneration of the cut fibres causes an increase of excitability in the nerve cells in which they end. Degeneration of peripheral nerves has been shown to cause increased excitability to drugs. The greater excitability of the tissues, as that of the spinal cord, is of gradual development and the greater number of fibres degenerating a little below the place of section of the cord than more peripherally might account for its greater excitability in the former region.

Note on the action of strychnine on autonomic nerve centres.

It has incidentally been mentioned above that afferent nerve stimulation after injection of strychnine has no reflex effect on the hairs, and no very obvious effect on the abdominal or pelvic viscera. Corresponding with this absence, or merely slight increase, of reflex excitability after strychnine has been injected is an absence of stimulation, or a merely slight stimulation, on its injection. It causes slight contraction of the bladder, and may cause some contraction or inhibition of the intestine, but it does not cause in the curarised spinal or decerebrate animal micturition or defæcation, nor any prolonged inhibition, or tonic contraction of the intestine. In the spinal cat I have not found that strychnine causes secretion of sweat, but my observations have been incidental; it is certain however that it may cause large rises of blood-pressure without any secretion. Pillcher and Sollmann(22) found that it caused contraction of the spleen, but the contraction was apparently slight. Acceleration of the heart in spinal cats generally occurs when strychnine causes a sudden rise of blood-pressure, but this appears to be due mainly to increased pressure acting on the heart, for in more gradual rises of pressure, the rate of heart beat is altered little or not at all. Whether the primary flushing which strychnine causes in the foot of the cat is due to stimulation of vaso-dilator sympathetic nerve cells I hope soon to decide.

Strychnine does apparently stimulate the sacral vaso-dilator nerve cells. To investigate this, a few experiments were made on decerebrate and curarised cats, the penis being freed from the prepuce and surrounding tissues. The injection of 2 to 5 mg. of strychnine caused marked swelling and protrusion of the penis. Subsequent injections (5 to 20 mg.) had a variable effect. One experiment was made on a dog.

In this the primary effect of the first dose was retraction of the penis, although the bucco-facial region flushed strongly.

In the anaesthetised cat, strychnine causes moderate primary dilatation of the pupil and other sympathetic eye effects. Section of the cervical sympathetic delays the action, suggesting that the primary effect is due to weak sympathetic stimulation.

Schlesinger⁽⁶⁾ describes strychnine as causing strong contraction of the uterus in the rabbit. Apart from this possible exception (and the reaction in the rabbit may differ from that in the cat), a *strong* stimulating action of strychnine on spinal nerve cells is, in the cat, confined to those governing striated muscles and blood vessels.

On the autonomic centres above the spinal cord the stimulating action is also limited.

In the cat and dog, after extirpation of the superior cervical ganglion to prevent pupil dilatation by way of the sympathetic, strychnine does not cause contraction of the pupil, so that the pupillo-constrictor centre (tectal autonomic) is not appreciably stimulated.

An action of strychnine on some bulbar autonomic centres is open to doubt. It occasionally causes secretion of saliva, but commonly it does not; when secretion occurs it is possibly a reflex produced by the bitter alkaloid stimulating the taste buds. S. Mayer⁽⁴⁾ considered that it stimulated the cardio-inhibitory centre. What he found was that with the rise of blood-pressure there was sometimes slowing of the heart. In decerebrate and curarised cats slowing of the heart is inconstant, it occasionally occurs after section of the vagi (and also in spinal animals) when there is a sudden rise of blood-pressure; at any rate the inhibitory action is slight. Here again the only strong stimulation appears to be of the somatic and vaso-motor mechanism.

The proof given by Wertheimer and Delezenne that strychnine stimulates the bulbar vaso-dilator centre I have mentioned in an earlier paper (⁽¹⁹⁾, p. 157). In this statement I overlooked the experiments of Dubois (*C. R. Soc. Biol.* 1904, Pt I, p. 355) who found that flushing of the tongue of the dog caused by strychnine after injection of adrenalin was prevented by section of the lingual nerve.

SUMMARY.

The observations except some of those referred to in paragraph 9 were made on curarised spinal cats. The animals were anaesthetised first with chloroform then with C.E., then either decerebrated, the vagi cut and the cervical spinal cord severed, or they were decapitated.

1. A trifling rise of blood-pressure of 1 to 4 mm. Hg can usually be obtained by stimulating the central end of any limb or trunk nerve. Occasionally the rise is somewhat greater. It is less constant in eviscerated than in non-eviscerated animals, and in hungry than in digesting animals. It is concluded that in normal body conditions afferent somatic nerves are capable of causing a trifling rise of blood-pressure as a spinal reflex.

2. A rise of blood-pressure is constantly obtained, as noticed by Sherrington, by stimulating the central end of a splanchnic nerve. In the experiments the rise varied from about 8 to 24 mm. Hg (once 33 mm.).

3. Stimulation of the central end of a hind limb nerve sometimes causes faint flushing followed by faint pallor in the hind feet without observable change in the fore feet. When the abdominal viscera are exposed in warm Ringer's fluid the hind limb nerves appear to cause slight pallor and other effects in the large intestine, and the brachial nerves to cause pallor in the stomach and duodenum. It is concluded that the reflex effect is restricted in area, but that it is not so restricted as to make it of importance in local circulation.

4. In all cases in which the excitability of the spinal cord is not greatly lowered, a reflex rise of blood-pressure can be obtained after injecting strychnine. The extent of the rise depends on the amount of strychnine. Most of the experiments were made on cats fed the night before. On these the following results were obtained. An amount of strychnine which caused only slight rise of blood-pressure usually enabled the sciatic to cause a rise up to 20-40 mm. Hg. An amount of strychnine not more than sufficient to cause a large rise of blood-pressure after a delay of a minute or more enabled stimulation of the sciatic or of the brachial nerves to cause a large rise of blood-pressure. In both of these stages, the rise decreased with successive stimulations. An amount of strychnine just sufficient to cause an approximately maximal rise of blood-pressure, enabled a somatic nerve, or the splanchnic, to cause a rise about double that obtained before strychnine was given. Further increase in the amount of strychnine decreased the reflex rise and eventually prevented its occurrence. In spinal animals in full digestion much greater rises of blood-pressure were obtained in all stages of increase, especially in the first.

5. After a small amount of strychnine, reflex changes in the colour of the feet are great. In hungry animals pallor of all the feet was the predominant effect, but sometimes there was some primary flushing, greatest in the hind foot of the side opposite to that on which the sciatic

was stimulated. In the animals in full digestion, primary flushing was greater and more constant, sometimes it continued during the whole period of rising blood-pressure and for a short time after the pressure had begun to fall. It was greatest and lasted longer in the hind foot on the opposite side to that on which the sciatic was stimulated. In all cases it was succeeded by pallor. In the foot of the same side the stimulation caused retarded and less primary flushing followed by maximal pallor although the anterior crural nerve as well as the sciatic was cut.

6. Any considerable reflex rise of blood-pressure after giving strychnine was accompanied by pallor of the whole of the exposed intestine, but after a certain time of exposure, large spontaneous rises of pressure occurred without appreciable pallor of the intestine. Reflexes on visceral movement were apparently only slightly affected by strychnine.

7. No reflex effect from the limb nerves or from the splanchnic was found either before or after strychnine on the hair or the sweat glands. The upper lumbar nerves in rare cases caused local erection of hairs, probably in consequence of an asphyctic increase of excitability of the spinal centres in the particular experiments.

8. The great reflex rise of blood-pressure obtainable after a small amount of strychnine has been injected makes it probable that in pathological cases of increased spinal excitability, spinal vascular reflexes are important. It is suggested that the increased response of certain autonomic spinal centres which occurs a week or more after section of the spinal cord is due to an increase of excitability in the nerve cells caused by degeneration of the descending nerve fibres ending in them.

9. No evidence was obtained that strychnine greatly increases the excitability of any autonomic nerve centre except the vaso-motor centres.

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TWENTY-FOUR HOUR OBSERVATIONS ON THE METABOLISM OF NORMAL AND STARVING SUBJECTS

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THE observations about to be described form a further part of a series of investigations into the metabolic activities over given periods. They differ from those already published in that the observation time is much longer—24 hours. Working with short periods, one of us (E C D (1)) demonstrated that there were more or less definite relations between the alveolar and blood CO_2 tension, gastro-intestinal secretion (2), and certain urinary constituents (3). These relations were altered by the effect of meals.

The results of the previous investigations can be summarised briefly as follows:

1 During a period of starvation, the alveolar CO_2 tension remains more or less constant, whilst the urinary pH and A + N value do not show any definite change.

2 After a meal, the alveolar CO_2 tensions show the characteristic rise followed by a fall. The urinary changes were found to vary according to the meal. Thus the breakfast was followed by a definite alkaline tide, as shown by a rise in the pH and a fall in the A + N value. This alkaline tide was usually of short duration, and was followed by a prolonged acid tide, as shown by a fall in the urinary pH and a rise in the A + N values—both of which obscured any alkaline effect of the next meal.

It was felt that these investigations were incomplete, since the observation times were never longer than 8 hours, and only the pH and A + N values were determined. The present investigation was undertaken, therefore, in order to clear up the difficulties of the previous observations.

Variations in the composition of urine throughout 24 hours have been studied by many observers. Thus Bence Jones (4) was perhaps the first to notice the alkaline tide following a meal. Since this observer's

¹ On behalf of the Medical Research Council.

time, many publications have appeared dealing with this subject. Some supported Bence Jones' original hypothesis (Campbell⁽⁵⁾, Fiske⁽⁶⁾, Dodds), whilst others have either partially (Leathes⁽⁷⁾) or completely (Quincke⁽⁸⁾) denied the dependence of the alkaline tide upon meals. As pointed out in a previous communication, the majority of previous observers neglected the following points:

1. They assumed that all their subjects possessed normal alimentary secretions. This is not justifiable since many perfectly healthy people are achlorhydrics. Thus Bennet and Ryle⁽⁹⁾ found 4 p.c. of achlorhydrics amongst normal students.

2. The acid tide of the breakfast is usually at its height just at lunch time, consequently the alkaline tide due to that meal is obscured.

3. The observation periods were too short and no rigid starvation controls were performed.

It was decided that 48 half-hourly observations for 24 hours would overcome the majority of these difficulties. Five subjects were selected, three of whom took food at the usual time, and two who acted as starvation controls. Since Izod Bennett⁽¹⁰⁾ had shown that water may cause gastric secretion, the starvation controls refrained from drinking throughout the period of the experiment. Sleep was, of necessity, an impossibility. Any results obtained from these observations are not without somewhat serious objection. Thus it can be argued that the subjects were being examined under pathological conditions, since no sleep was obtained. It is suggested that conclusions based on half-hourly specimens of urine are of greater value than any observations on the whole of the night urine obtained when the subject wakes in the morning. Also the methods adopted by some observers, of waking the subject every 2 hours seemed to us more disturbing than going without sleep entirely.

Methods. The subjects were five healthy men working in the Biochemical Department. The gastric and pancreatic secretions of all had been previously proved to be normal either by the fractional test meal method, or by the CO₂ method of examination.

The subjects dined at 7 P.M. and the experiment was commenced at 9 P.M. The subjects emptied their bladders at the commencement of the observation, and then at half-hourly intervals through the following 24 hours. The specimens of urine were preserved for analysis with toluol. Specimens of alveolar air were collected and analysed at hourly intervals throughout the period (by the Haldane-Priestly⁽¹¹⁾ method).

As soon as each specimen of urine was passed, the volume was measured, and the whole was poured into a flask containing a small

quantity of toluol. The specimens were analysed as soon as possible although it can be realised that the examination of 240 specimens takes up a fair amount of time. The following series of estimations was carried out

- 1 Volume
- 2 The hydrogen ion concentration as determined by indicators
- 3 Titrable acidity and ammonia, by Malfatti's method⁽¹²⁾. As only small quantities were available, a micro method, using a comparator, was employed. This method was used by one of us (E C D) in a previous investigation of a similar character, where it was found to be perfectly satisfactory. To 5 c c of urine 20 c c of boiled distilled water was added, followed by 0.75 c c of cresol red as an indicator. Neutral potassium oxalate (10 gm) was added in order to precipitate those salts which tend to obscure the end-point. A standard solution of pH 7.4 was prepared by means of Sorensen's buffer phosphate solutions, and 0.77 c c of the cresol red solution added. The resulting solution was of a brownish colour, and was placed in a comparator. The urine was titrated to the colour standard by the addition of *N*/10 acid or alkali, according to whether it was acid or alkaline. All comparisons were made in the comparator.

The titrable acidity having been determined, the ammonia content was arrived at by Malfatti's (1908) formalin method. To the solution obtained above 10 c c of 20 p c formalin were added, the formalin having been neutralised in the manner described for the estimation of titrable acidity. The resulting acidity is then titrated as before.

- 4 The total nitrogen was determined by Folin's⁽¹³⁾ micro Kjeldahl method. Owing to the very small volume of many of the specimens, the solutions resulting from the estimation of ammonia and acidity were used for this purpose after suitable dilution. This solution originally contained 5 c c of urine, and, since all the reagents added were nitrogen free, with the exception of the cresol red, the quantity of which was constant in all specimens, there can be no objection to this process.

- 5 The urea content was estimated by a method similar to Folin's technique⁽¹⁴⁾ for the estimation of urea in blood. 0.5 c c of urine was diluted to 400 c c. 5 c c of this mixture was pipetted off, and the reaction was adjusted to about pH 6, by using brom cresol-purple as an indicator. The method was then carried on as in Folin's estimation of urea in a protein free filtrate by digestion with urease paper and subsequent distillation and nesslerisation. This method gives all the urea as ammonia nitrogen, plus the urinary ammonia nitrogen. Since the latter has

already been determined, the true urea nitrogen can be obtained by subtraction.

6. Inorganic phosphates, by Briggs' (15) modification of Bell and Doisy's (16) colorimetric method.

7. Diastase, by the colorimetric method described by us in the *British Medical Journal* (17). By this method it is possible to estimate the diastatic content to the nearest unit.

8. The deposit in each specimen was examined microscopically.

Calculation and expression of the results. The plan adopted by most workers of expressing the results as hourly excretion rates has been adhered to here. The calculations were simplified by maintaining the time interval, half-an-hour, between each specimen absolutely constant. Moreover, this short period tends to minimise the multiplication of errors in the calculation of hourly rates.

The ammonia and nitrogen "coefficients" were calculated in one of the normal and one of the starvation cases. These values are given by the following calculations:

$$\text{Ammonia "coefficient"} = \frac{\text{ammonia nitrogen per 100 c.c.}}{\text{total nitrogen per 100 c.c.}} \times 100.$$

$$\text{Nitrogen "coefficient"} = \frac{\text{urea nitrogen per 100 c.c.}}{\text{total nitrogen per 100 c.c.}} \times 100.$$

By this means it is possible to obtain some idea of the distribution of nitrogen.

The urea nitrogen, total nitrogen, and phosphates are expressed as mg. per hour. The titrable acidity and ammonia are recorded in the manner recommended by Henderson (18), the A + N value. This is given by the sum of the hourly rates of excretion of titrable acidity and ammonia expressed as c.c. N/10. The diastase is expressed as Wohlgemuth units per hour and the hydrogen ion concentration in the pH nomenclature.

Results. Owing to the large number of figures, the publication of tables is rendered impossible through the cost involved. The results have therefore been recorded as curves, which are reproduced. Only two of the normal curves are published. The time of the meals has been indicated by an arrow. It will be seen at a glance that the normal and starvation curves respectively are of the same type, and that they rise and fall more or less together. It will, perhaps, be as well to take each constituent in turn.

Volume. All the subjects excreted decreasing quantities of urine during the night from 9 P.M. to 8.30 A.M. After this time, the amount

excreted by the starvation controls remained more or less constant, and at a low level during the rest of the experiment. After taking the first meal, the three normal subjects first excreted less, and then more urine, the curve ultimately reaching a much higher level. This drop, followed by a rise in the volume of urine was also observed in a series of analyses described by one of us (E. C. D.) in an earlier investigation. The falling off in the first half hour following a meal seems to be associated with gastric secretion, and we have observed that it varied roughly with the amount of gastric secretion. Thus in one case, examined with Dr T. Izod Bennett, who performed simultaneous gastric analysis, the excretion of urine was practically reduced to one-quarter, half-an-hour after a meal. The patient had intense gastric hypersecretion, and proved to be a genuine case of Reishmann's disease. The amount of urine secreted during the day in the three normal cases was very much greater than during the night. The comparatively small volume of the night urine was noted as early as 1843 by Scheinig⁽¹⁹⁾, and was later confirmed by Vogel⁽²⁰⁾. Speck⁽²¹⁾ in 1881 stated that the increase during the day was due to the intake of fluids. Quincke⁽²²⁾ considered the increase during the day to be associated with activity, rather than with the intake of fluid. The curves reproduced here rather support the earlier view, since there is no increased excretion in the starvation controls, who worked hard in the laboratory throughout the 24 hours.

The hydrogen-ion concentration. Since this determination could not be performed at once, the accuracy of the results must of necessity fall under some doubt, consequently the results are not recorded graphically. The most noticeable point was that the pH of the starvation curves was found to be remarkably constant at or about pH 5 to 6, whilst in the feeding subjects there was extreme variation in the figures. The taking of the first meal was followed by a slight increase in the pH, thus supporting the previous investigations of Fiske, and one of us (E. C. D.). The lunch and tea effects were obscured by the acid tides following breakfast.

Ammonia and acidity. As already explained, these values have been expressed as A + N. The results obtained are in complete agreement with those of previous workers (Campbell, Dodds). There is a steady fall in the values from 9.30 P.M. to 8 A.M. After the first meal the curves fall still lower, but rise after a period of 1 to 2 hours. This alkaline tide is followed by an acid tide, as shown by a very sharp rise in all the normal curves. This acid tide lasts for about 3 hours, when lunch is taken. There is then a drop in all the curves, but not to the same extent as after breakfast. This second alkaline tide lasts for a little over an hour, when

the curves rise very sharply until tea is taken. Two of the normal curves show a very marked rapid fall at this point, whilst the third rises, to fall later.

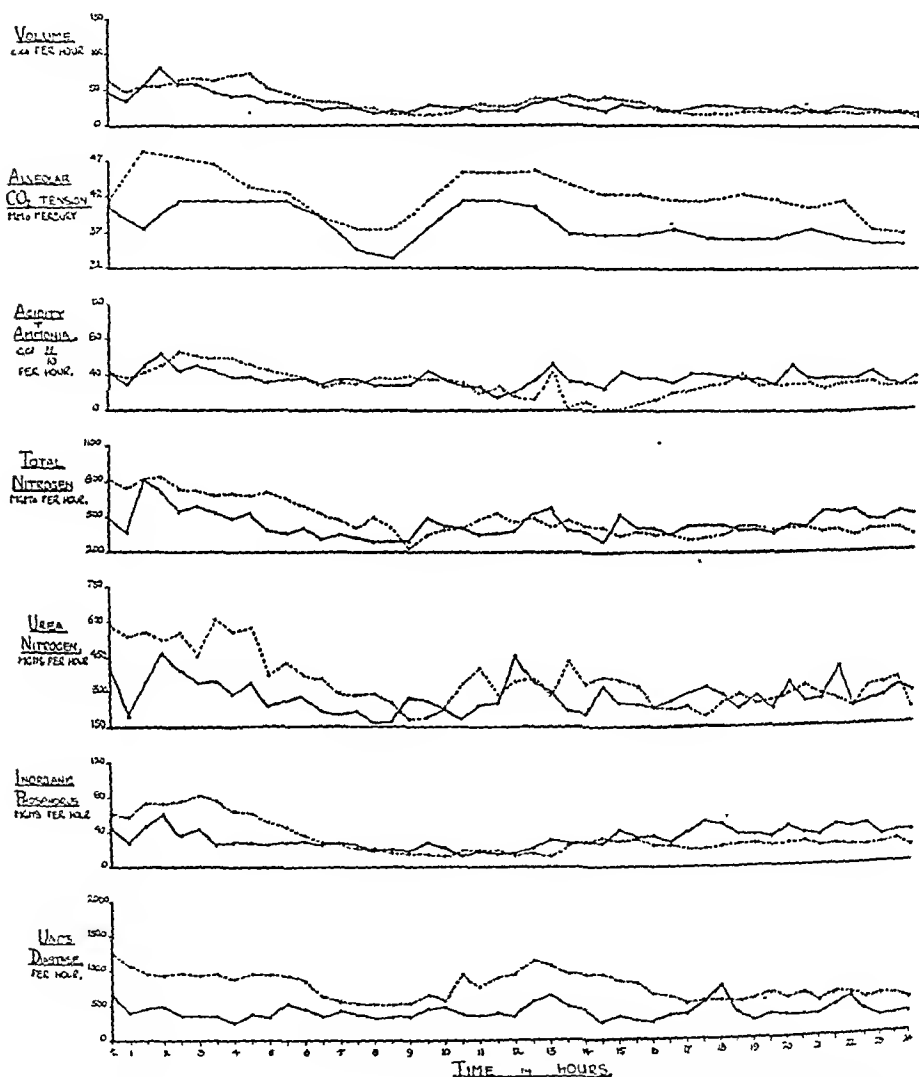


Fig. 1. Half-hourly analyses of urine in man without food for 24 hours. (Two subjects.) The experiment commenced at 9 P.M., the first urine being taken at 9.30 P.M. The 3rd and 15th hour of the experiments correspond to midnight and mid-day respectively.

In the starvation subjects, the ammonia and acidity excretion rates fall steadily until 9.30 A.M., i.e. for 12 hours. There is then a marked rise, which continues for the rest of the experiment. Moreover, the

curves are much smoother than the normals, and really consist of a steady fall for the first 12 hours, followed by a steady rise for the next 12 hours

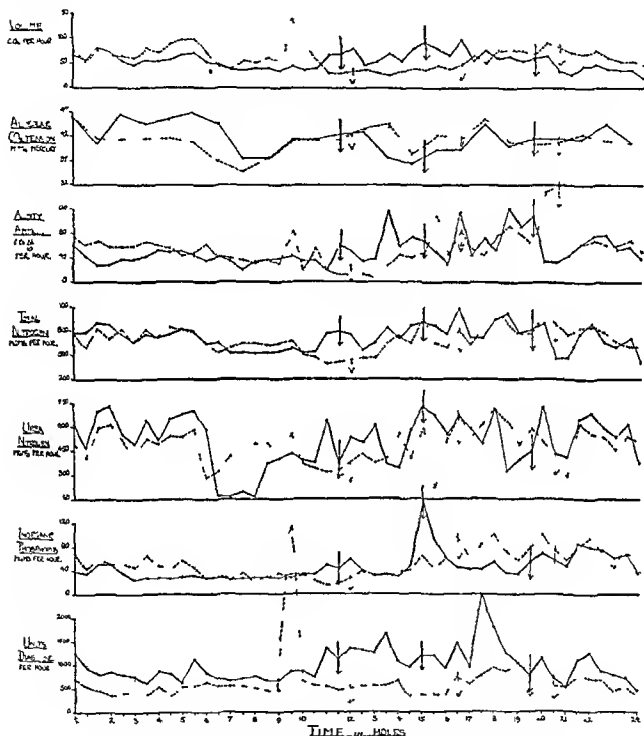


Fig 2 As Fig 1, but meals begun 11½ hours after being without food
The time of taking meals is shown by the arrows

The swing on the normal curves are of much greater amplitude than on the starvation charts, even up to as much as $2\frac{1}{2}$ times. From these observations it would appear that the large variations in the A + N value are entirely due to meals, and that they are not of diurnal origin.

The views of Leathes that there is a decided tendency to alkalinity in the early morning specimens is also supported, since there is a steady fall in the A + N curves from 9.30 P.M. to 5 to 7 A.M.

Urea. The rate of urea excretion fell slowly until about 3.30 A.M. in the normal subjects, after which it began to rise until the first meal. After this the rise is steeper, and the maximum level was reached at about 12 noon. The rate remained more or less constant until about 5 P.M., when the curves began to drop. In the starvation curves, the rate falls steadily until about 7 A.M. when there is a slight rise until 9.30 A.M. after which time the rate remains about constant. As would be expected, the curves are at a much lower level than those of the normal subjects. From an examination of the curves, it would appear that the increase in rate of excretion is due to meals. As the immediate effects of taking food are slight and variable, it would appear that the meals act as a whole, and not individually. The maximum rise appears to be about 3 hours after the first meal.

A great deal of work has been done in the factors governing the rate of urea excretion. Ambard⁽²³⁾ maintained, after many careful experiments, that the excretion depended upon the blood urea, and from his observations, founded his well-known theory which he expressed in mathematical form. The essential part of Ambard's coefficient depends upon the assumption that the amount of urea excretion increases as the square root of the blood urea. In other words, were the blood urea to be doubled, the excretion of urinary urea would be quadrupled. This mathematical expression has been criticised by many workers, more especially by Marshall and Davis⁽²⁴⁾, who stated that provided sufficient water was given, the excretion of urea varied more or less in direct proportion to the blood urea. Addis and Watanabe⁽²⁵⁾ maintained that the rate of excretion of urea could be increased by increasing the volume of urine, but "there is no evidence that this increased rate is a result of the increased volume of urine for the degree of increase above normal in the rate is quantitatively independent of the degree of increase in volume." Moreover, they also state that their results "cannot be accounted for on the basis of changes in blood urea concentration." Although we were unable to estimate the blood urea at frequent intervals, we have every reason to believe, from other observations, that the variations in this value are very slight. The table given below shows the range of blood urea and non-protein nitrogen contents, together with certain other constituents over a period of 4 hours of four normal and two starving men.

	Time	Urea	Non protein nitrogen	Uric acid	Creatinin	Amino nitrogen	Sugar	Chlorides
No 1 Meal between 12 and 1	11 A M	30	35	3	1 5	6	106	470
	12	30	37	3 5	1 5	5 9	109	470
	1	28	29	3	1 5	5 9	116	480
	2	33	30	4	1 5	6	121	477
	3	30	32	4	1 4	6	120	480
No 2 Meal at 1 P M	2 P M	21	26	2	1 2	5	126	491
	3	23	26	3	1 2	4	118	490
	4	22	28	3	1 3	5	121	496
No 3 Meal at 11 30 A M	9	28	32	4	1 4	5	121	486
	10	31	34	3 5	1 4	4	131	510
	11	36	32	4	—	—	—	—
	12	32	31	4	1 3	6	132	508
No 4 Meal at 1 A M	12	27	31	3 2	1 4	6	121	510
	2	36	37	4 6	1 4	7	131	509
	4	28	30	4 2	1 4	7	135	510
No 5 Starvation	12	25	30	3 2	1 5	6	100	481
	2	25	36	3 8	1 4	4	92	480
	4	23	31	3 9	1 4	5	98	480
No 0	9	29	28	4 0	1 3	7	110	501
	12	31	29	4 0	1 2	7	100	504
	3	30	27	4 0	1 1	0	89	500

From the figures in the table, expressed as milligrams per 100 c c of blood, it can be seen that there is very little swing in any of the values. Folin(20) had previously called attention to this. Since our urinary figures show marked variation, even up to 200 p c, it seems obvious that these changes cannot be ascribed to fluctuations in the blood urea concentration. This bears out the work of Addis and Watanabe previously referred to.

Total nitrogen The excretion of nitrogen falls steadily throughout the night in all the subjects. The starvation controls then continue to excrete, at a roughly constant rate, from 7 30 A M to the end of the experiment. After the first meal all the normals show a marked rise within 2 hours. The curves rise until lunch, and continue at a high level until 5 30 P M. All the curves then began to fall. The curves are very similar to those for urea. The explanation must lie in the fact that nitrogenous products of digestion and absorption reach the urine within 2 hours. The effect of breakfast, lunch and tea are merged into the rise lasting from 9 30 to 5 30. At 8 30 the curves rise again due to the diurnal effect, which is seen at the beginning of the charts.

The diastase It has been stated by many observers that there is a rise in diastatic index of the urine following a meal, but no details are available as to the diastatic content over a period of starvation. An examination of the curves provided proves that variations occur quite

independent of the intake of food. Thus, although the diastatic power of the urine shows a more or less definite rise after meals, the starvation curves show elevations at the same points. Moreover, the rise in the starvation curves is of the same magnitude as in the normal. Judging from the irregularity of all the curves, it would appear that the excretion of diastase is quite independent of gastro-intestinal secretion, and probably follows irregular absorption from the gut. The curves demonstrate the uselessness of placing confidence for diagnostic purposes on estimations performed on isolated, short-time specimens of urine. Also, since such enormous variations occur during health, it is inadvisable to regard any slight deviation from the normal with significance.

Inorganic phosphates. All the curves show a steady fall up to breakfast time (8 A.M.). The starvation controls then show a very slight rise, after which the curves remain at a more or less constant level up to the end of the experiment. Practically every meal is followed by an immediate diminution in the excretion of phosphates, which in turn, is followed by a rise about 1 to 2 hours later. These variations must be due to the meals, since they are absent in the starvation controls.

The alveolar CO₂ content. The curves are irregular, owing to the fact that it was only possible to perform analysis at hourly intervals, and consequently the rapid changes following a meal are obscured. The curves of the normal subjects are irregular, but the rises and falls correspond roughly with the changes already described. In the starving subjects, there is a steady fall until the end of the experiment. This is in agreement with the results of Haldane and Fitzgerald (27).

The ammonia and nitrogen coefficient. These values were worked out and plotted in a starving and in a feeding subject. By this means it was hoped to detect changes in the nitrogen distribution between ammonia and urea. The generally accepted view is that the two values vary inversely, i.e. if the percentage of ammonia nitrogen increases, there will be a corresponding decrease in the urea nitrogen percentage, because urea is formed from ammonia. An examination of the starvation curve shows that the two values rise and fall more or less together, and that this inverse ratio does not hold over short spaces of time. When the effect of the dinner, previous to the experiment, has worn off, both curves show only very slight fluctuations, and move more or less together. The state of affairs in the feeding subjects fits in more closely with the generally accepted views. Thus, after a meal, there is a drop in the ammonia coefficient, and a rise in the nitrogen coefficient. This corresponds with the alkaline tide. There are much greater swings on the

normal than the starvation curves, and the two values vary more or less inversely. The figures from the other three subjects are similar to the above.

SUMMARY AND REMARKS.

The urine of five normal men, three taking food, and two starving, was examined at half-hourly intervals for 24 hours. The object of the investigations was twofold. Firstly to determine the half-hourly variations of the urinary constituents, and, if possible, to assign to them some cause; secondly to determine to what extent exogenous factors operate. The results were incomplete in so far as it was found to be impossible, both from the point of view of labour and material, to estimate every urinary constituent. The results also lost value from the lack of simultaneous blood analysis; this factor cannot be overcome, since it is impossible to bleed an individual 48 times in 24 hours. The majority of the blood constituents remained remarkably constant over a given period. Folin, in his third Mellon lecture, stated: "In normal persons, ...there is no material change in the urea concentration of the blood because of changes in the level of the nitrogen metabolism." The Table giving our own analysis is in complete agreement with this statement, since the only blood constituent showing any marked variation is the glucose. *Our results could not be interpreted by the laws of Ambard.* It was surprising that the urea and nitrogen excretion were increased within 2 hours of the first meal in the day. After this rise, it was impossible to recognise the effect of individual meals, since the curves showed irregular plateaux from breakfast to tea time.

The evidence supplied by the curves seemed to support the view that an alkaline tide is associated with meals, and, more particularly, with gastric secretion. After breakfast, the following series of changes were found to occur: (1) a rise in the alveolar CO_2 tension, which has been shown to correspond with the secretion of HCl from the stomach; (2) a slight rise in the pH of the urine; (3) a fall in the $\text{A} + \text{N}$ value; (4) a fall in the ammonia coefficient, and (5) a rise in the nitrogen coefficient. These changes were all reversed a little later, when pancreatic secretion began to flow. That the later meals of the day were not followed by such clear cut changes, found explanation in the fact that the alkaline tide of such a meal was superimposed upon the acid tide of the previous meal. This evidence supports very strongly the original contention of Bence Jones, as the starvation controls in these observations were conducted under very rigid conditions. It is possible that the variations

in the phosphate excretion were also bound up in this problem. Investigations on these lines are proceeding.

Another interesting point is that evidence of acidosis was present even after so short a period of starvation as 24 hours. Thus the alveolar CO_2 tension showed a steady fall, and there was a distinct tendency for the ammonia coefficient to rise, and the nitrogen coefficient to fall.

The marked variations in the urinary diastase could not be attributed to meals, since marked variations occurred in the starvation controls. It would appear, therefore, that in the cases examined, the urinary diastase content was quite independent of the intake of food.

All the results emphasise the uselessness of analysing isolated specimens of urine, and of basing conclusions on short time observations without rigid starvation controls.

In conclusion, we have to thank the three volunteers who joined us in this somewhat irksome investigation. It is a pleasure to acknowledge the assistance of Miss Bridgwater and Dr Potts Kinnard of Philadelphia, in performing the large number of tedious analyses.

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INFLUENCE OF CO_2 ON THE ABSORPTION OF ALCOHOL BY THE GASTRIC MUCOSA.

By NORA EDKINS AND MARGARET M MURRAY

(From the Physiological Laboratory of Bedford College, London)

It is known that alcohol is one of the substances which the stomach does absorb, also it is generally held that alcohol in the presence of "aerated" water which contains a high concentration of CO_2 is absorbed more rapidly than alcohol diluted to the same extent with water. Although a fair amount of work has been done on the absorption of alcohol in the alimentary canal as a whole, especially by Mellanby⁽¹⁾, apparently no investigations have been published dealing with absorption restricted to the stomach.

Method Cats only were used. The animals, after preliminary anaesthesia with chloroform, were decerebrated by Langley's method¹, and, throughout the experiments, were completely immersed in a normal saline bath kept constant at 37°C . The oesophagus was tied, care being taken not to include obvious blood vessels, an incision made into the duodenum just beyond the pylorus and a T tube, fitted at one end with a stopcock, introduced through the pylorus into the stomach. Liquid could then be introduced through one limb and removed by the stopcock. In all cases the animals were fasting and the stomach was well washed out with unbuffered Ringer's solution. The amount of liquid introduced was approximately 60 c.c.

As a preliminary to the investigation of the influence of CO_2 on the absorption of alcohol by the gastric mucous membrane, some experiments were carried out to determine whether the medium in which the alcohol was later to be dissolved was itself absorbed. For this purpose a known volume of unbuffered Ringer's solution was introduced into the stomach, left for one hour, removed, measured and analysed for hydrochloric acid.

J. S. Edkins⁽²⁾ found that there was no absorption of water in the stomach of cats under chloroform anaesthesia, but since in all abdominal experiments it is generally agreed that a nearer approach to the living

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As a preliminary to the investigation of the influence of CO₂ on the absorption of alcohol by the gastric mucous membrane, some experiments were carried out to determine whether the medium in which the alcohol was later to be dissolved was itself absorbed. For this purpose a known volume of unbuffered Ringer's solution was introduced into the stomach, left for one hour, removed, measured and analysed for hydrochloric acid.

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¹ Injection into the distal end of carotids a 10 p.c. suspension of starch grains in 7 p.c. gum Ringer to keep the starch in suspension.

condition is obtained in the decerebrate preparation, it remained to be seen whether such a physiologically inert liquid as Ringer's solution would be absorbed or excite secretion in this state. The apparatus was similar to that used by Edkins and the liquids were introduced and kept at a pressure within the stomach of 2-5 cms. H_2O . The results (Table I)

TABLE I. Absorption of unbuffered Ringer.

Exp.	C.c. of Ringer introduced into stomach	C.c. of Ringer removed after 1 hour from stomach	Absorption	Grm. HCl. secreted
1.	84.2	85	-0.8	.007
2.	75.9	73	+2.9	.001
3.	81.5	82.5	-1	.003
4.	91.4	93	-1.6	.018
5.	64.9	65	0	.01
Average exp. 6-10	57	—	- .7	—

showed that since the differences between volumes introduced and withdrawn were practically no greater than ± 1 p.c., there is no appreciable absorption. There was, however, usually a small secretion of HCl. For comparison several experiments were performed in which Ringer's solution was introduced into loops of small intestine, here the liquid was always entirely absorbed within 20 minutes.

Absorption of alcohol and influence of CO_2 . After washing out the stomach, the diluted alcohol (which in most of the experiments was a 5 p.c. solution in unbuffered Ringer) was introduced. Each experiment consisted of two parts:

- (a) the absorption of alcohol in the Ringer free from CO_2 ;
- (b) the absorption of alcohol in the Ringer containing a high concentration of CO_2 .

Each part of the experiment was allowed to run for one hour and since the second part might be considered a "fatigue" experiment the order of (a) and (b) was alternated in successive experiments.

To obtain evidence upon the influence of CO_2 on the rate of absorption of alcohol (Table II), liquid was withdrawn immediately after introduction and at 20-minute intervals throughout the experiment, each sample was analysed for alcohol and CO_2 content. The alcohol was estimated by the Cannan and Sulzer modification (3) of Pringsheim's method. The CO_2 was estimated by withdrawing liquid from the stomach under the surface of a known volume of baryta water in a cylinder and titrating excess baryta with oxalic acid. This gave $CO_2 + HCl$. A known volume of the liquid was boiled out, a known amount of baryta water added,

TABLE II Rate of absorption of alcohol in presence or absence of CO₂
(a)=No CO₂ present (b)=With CO₂ present

Exp		CO ₂ at begin ning %	CO ₂ at end of 1 hour %	Conc alcohol at time 0 (intro duction) %	Conc alcohol after 20 min %	Conc alcohol after 40 min. %	Conc alcohol after 60 min %
1	(a) 1st hour	None	None	4.38	3.83	3.34	2.61
	(b) 2nd "	66	4.4	4.41	3.77	2.74	2.31
2	(b) 1st hour	50	None	4.63	3.68	2.20	1.55
	(a) 2nd "	None	None	4.44	4.26	3.53	2.41
3	(a) 1st hour	None	2.6*	4.77	3.56	2.33	1.75
	(b) 2nd "	52.4	None	3.8	3.53	1.93	1.22
4	(b) 1st hour	46.4	None	9.36	5.35	3.46	2.10
	(a) 2nd "	None	None	7.11	6.87	5.23	3.46

* This was about the only case where CO₂ initially absent was found to have diffused out from the gastric mucous membrane into the stomach cavity

the excess titrated with oxalic. The difference between these titrations gave the CO₂ while the difference between the last titrations and the standard titrations for the baryta gave the HCl. This last value was checked by titrating the boiled out liquid with N/100 NaOH.

Another series of experiments was performed to determine the quantity of alcohol absorbed throughout the time of the experiment. In this (Table III), liquid was withdrawn immediately after introduction

TABLE III Absorption of alcohol in presence or absence of CO₂
(a)=No CO₂ (b)=With CO₂

Exp		Conc of CO ₂ at begin ning %	Conc of CO ₂ at end %	Volume of alcohol at begin ning c c	Volume of alcohol at end c c	Actual amount of alcohol absorbed c c	HCl secreted in mgm.
1	(b) 1st hour	42.6	None	2.17	8	1.67	36.7
	(a) 2nd "	2.5	None	1.84	6	1.24	48.94
2	(a) 1st hour	None	0.5	1.79	39	1.4	21.5
	(b) 2nd "	76.8	2.0	1.53	43	1.11	31.1
3	(a) 1st hour	0.45	None	2.24	5	1.74	3.79
	(b) 2nd "	51.8	None	2.13	52	1.66	37.85
4	(b) 1st hour	46.5	1.3	2.43	71	1.72	7.6
	(a) 2nd "	0.6	None	1.97	1.03	0.94	0.38
5	(a) 1st hour	0.6	None	2.07	56	1.51	2.96
	(b) 2nd "	40	None	2.38	67	1.7	4.74
6	(b) 1st hour	42.0	None	2.5	68	1.7	27.0
	(a) 2nd "	3.3	2.8	2.53	1.09	1.46	11.5

Concentration of alcohol solution in Exps 1, 2, 3 roughly 5% (actual amount estimated) and in Exps 4, 5, 6 roughly 6%.

In each experiment the difference between the liquids introduced was solely in CO₂ content.

and after an interval of one hour. Since we generally found no CO_2 in the liquid removed, regardless of whether CO_2 was originally present or not, we endeavoured to determine whether the presence of alcohol influenced the rate of disappearance of CO_2 from the liquid when present in large quantities. Similar experiments to those described were performed with no alcohol present in the liquid.

Exp.	CO_2 at beginning %	CO_2 at end %	HCl secreted mgm.
1. (a) 1st hour	0.23	0.5	0.4
(b) 2nd "	55.2	8.2	0.34
2. (a) 1st hour	None	2.3	3.4
(b) 2nd "	67.2	16.0	0.36
3. (b) 2 hours	48.2	3.6	3.9

These results show that though there is considerable loss of CO_2 from the liquid, the gas never disappears so completely as in the alcohol experiments; moreover, there is a tendency for the CO_2 content of the stomach liquid to equilibrate with that of the CO_2 tension of the gastric mucous membrane. It was shown by one of us⁽⁴⁾ that CO_2 in quantities not differing greatly from the normal amount will diffuse until an equilibrium is established.

DISCUSSION AND SUMMARY.

From the experiments, we can conclude that the presence of CO_2 in the alcoholic solution introduced, hastens the rate of absorption of alcohol by the stomach. The total amount of alcohol absorbed in either part of an experiment, *i.e.* (a) in the absence of CO_2 or (b) in the presence of CO_2 , depends on which part occupies the first hour. In cases where the CO_2 was present in the second hour, the absorption closely approximated to that of the first hour of the same experiment, on the other hand, if CO_2 was present in the first hour, the absorption of alcohol during the second hour, in the absence of CO_2 , was distinctly less than in the first hour. Apparently the alcohol does not pass through the gastric mucous membrane accompanied by H_2O since that would involve a considerable diminution in volume of the liquid in the stomach, whereas practically no alteration in volume occurs. The presence of alcohol also causes the CO_2 to disappear more rapidly and completely and prevents the liquid in the stomach equilibrating with the CO_2 tension of the tissues.

The passage of alcohol in some way profoundly changes the character of the mucous membrane as a diffusion membrane for the transit of CO_2 . This gas passes from the cavity into the tissues very rapidly and completely

in the presence of alcohol. It even seems to pass from a region of lower to one of higher tension, since it disappears entirely from the liquid in the cavity. Moreover, since an equilibrium is not established between the stomach liquid and the tissues, with respect to CO_2 , it appears that the gas is barred from passing out to the cavity from the mucous membrane.

There is no reason to believe that the living condition of the mucous membrane is abolished, nevertheless, the effect of the alcohol is to cause what may be regarded as a diffusion membrane to be permeable in one direction only.

This effect is shown well in the following experiment: Unbuffered Ringer containing a concentration of CO_2 , approximately equal to the gaseous tension of arterial blood was left in the stomach for one hour, the concentration of CO_2 rose to 4 p.c. This liquid was withdrawn and replaced by a similar solution of Ringer + CO_2 containing alcohol, the concentration of CO_2 in this solution fell below 1 p.c. in the hour. This effect of driving CO_2 from a region of low tension to one of higher tension is certainly remarkable, it is, however, transient, probably dependent on the simultaneous absorption of alcohol.

The fact that alcohol does (a) prevent the passage of CO_2 from the tissues into the stomach, also possibly the intestine, and (b) causes a very rapid absorption of CO_2 from the stomach liquid, must be borne in mind when alcohol is taken with aerated waters in metabolic experiments based on respiratory exchange.

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THE INFLUENCE OF THE OVARY ON PITUITARY SECRETION; A PROBABLE FACTOR IN PARTURITION.

By W. E. DIXON AND F. H. A. MARSHALL.

(From the Pharmacology Department and the School of Agriculture, Cambridge.)

"We may be said to be in the dark," wrote Michael Foster, "as to why the uterus, after remaining for months subject to futile contractions, is suddenly thrown into powerful and efficient action and, within, it may be, a few hours or even less, gets rid of the burden which it has borne with such tolerance for so long a time. None of the hypotheses which have been put forward can be considered as satisfactory." Williams has arranged these hypotheses under twelve heads and has briefly discussed each of them. He remarks that most of them are "extremely unsatisfactory" and "none is of universal application." "On the other hand, it is possible that some law may be discovered in the future which will explain the rhythm of the various sexual functions in women—menstruation, as well as the onset of labor."

There are, however, certain considerations which are the outcome of recent researches on the condition known as pseudo-pregnancy and which have a direct bearing on the problem as to the cause of parturition. The animals in which pseudo-pregnancy is known to occur are the marsupial cat (Hill and O'Donoghue), the opossum (Hartman), the dog (Marshall and Halnan), and the rabbit (under experimental conditions—Ancel and Bouin, 1910, and Hammond, 1917, etc.). In each of these species the corpus luteum persists during pseudo-pregnancy in the same kind of way as it does during true pregnancy, and the uterus and mammary glands undergo growth in correlation with the growth of the corpus luteum. In the rabbit which normally only ovulates after coition, the condition of pseudo-pregnancy can be induced by permitting the doe to have a sterile coition (as with a vasectomised buck). The corpus luteum, after being apparently functionally active, eventually undergoes involution, and in association with this process the uterus also regresses and the mammary glands begin to secrete milk. The same sequence of events occurs also in the other animals mentioned, but in these the processes

may be spontaneously initiated, that is, without coition. Moreover, at an advanced stage of involution which may be considered as marking the termination of pseudo-pregnancy the animals commonly display habits or instincts which are normally associated with the act of giving birth. Thus the bitch may prepare a bed as if for a litter of pups, the marsupial cat cleans out her pouch as if for the reception of young, and the doe rabbit plucks her breast of fur which she uses to line a nest. Since these habits are displayed at the end of pseudo-pregnancy which, as we have seen, is dependent upon the persistence of the corpus luteum, it is not unreasonable to suppose that the processes associated with actual parturition after true pregnancy are similarly correlated with ovarian changes depending upon the degree of involution of the corpus luteum and not solely upon the presence of the full-time foetuses. We have a definite hint here that the cause of birth is a function of a certain stage in the ovarian rhythm (Marshall).

Ancel and Bouin (1912) have suggested that the tolerance of the uterus towards the foetus during the first part of pregnancy is due to the corpus luteum, but that in the latter part of pregnancy it is caused by a certain uterine gland which they call the myometrial gland. The existence of the latter, however, has not been confirmed (Hammond, 1917) and the authors have formulated no definite hypothesis as to how these glands respectively act.

There is no clear evidence that the ovary has any direct influence in bringing about parturition. Such experimental observations as have been made on the effects of ovarian extracts on uterine contraction are conflicting and cannot be brought into any intelligible relation with the phases of the oestrous cycle or of pregnancy. For instance, Itagaki states that extract of corpus luteum generally produces an increase of tone in the uterus, but sometimes the opposite effect. Liquor folliculi was found to cause increase of tone both in uterine and also in other muscles. Further, it is not clear that the augmentor effect (when present) may not be partly or wholly due to the serum constituent or to histamine-like bodies. We have obtained evidence to show that ovarian extract and extract of corpus luteum act upon the isolated uterus in no way differently from extracts of other tissues. The isolated uterus suspended in Locke's fluid is so sensitive to trifling alterations in the composition of the fluid that the utmost care is required in drawing conclusions from experiments in which tissue extracts are added directly to such a preparation. On the other hand, it is well known that pituitary extract has a definite specific effect on uterine muscle. This fact has been taken advantage of in order to

standardise the strength of pituitary extract, the virgin uterus of the guinea-pig being used for this purpose (Burn and Dale). Moreover, pituitrin is now used by obstetricians to expedite parturition, more particularly in cases of difficulty. There is some suggestion, therefore, that the pituitary may be the source of the stimulus for labour, but the question remains as to why the pituitary should become activated at such a time, or what is the nature of the relation (if any) between the action of that organ and the rhythmic changes of the ovary.

Certain observations recently described by Dixon have suggested the solution of this question. As a result of a considerable series of experiments designed to test the influence of commercial ovarian and other extracts upon the secretory activity of the pituitary gland, Dixon found that ovarian extract had an exciting effect. Extracts of testis, epididymis, pancreas, etc., were used as controls, and with all of these the results were entirely negative. Commercial extract of corpus luteum was also absolutely negative. The method of experiment has been already described by Dixon. Briefly stated it consists in tapping the cerebro-spinal fluid in an anæsthetised dog and the subsequent injection into the circulation of the extract to be tested. Samples of the cerebro-spinal fluid subsequently secreted are then taken at short intervals and tested for pituitrin on the virgin uterus of a guinea-pig suspended in Ringer's fluid, the results being recorded in the usual way. If the result is positive it indicates that the extract employed has had a stimulating effect on the pituitary and caused secretion by that gland. The results of Dixon's experiments suggested that whereas normal ovarian secretion acting through the pituitary promoted uterine contraction, this action was inhibited during pregnancy by the corpus luteum.

In the experiments described in this paper extracts of whole ovaries were employed, and everything was known as to the precise stage of pregnancy or of the œstrous cycle at which the animals, whose ovaries were employed, were killed. In this way a fairly complete series was obtained, and it was found that the influence of the ovary upon the pituitary gland varied widely at different stages.

The first series of experiments was with rabbits, in which the average period of pregnancy is thirty-two days. The rabbits were killed by a blow on the head, the ovaries were extracted, minced and pounded up in a mortar with Ringer's solution and finally boiled and filtered. In some of the earlier experiments the extract was injected into the dogs unboiled, after merely filtering through glass-wool, but the result was the same. The nucleo-protein of the ovary is very toxic to dogs and this

was another reason why we preferred the boiled extract. The filtrate, generally consisting of about 20 c.c., was injected slowly into the femoral vein so that there was no appreciable effect on blood-pressure. C.S.F. was generally collected in three batches, (i) that secreted in the first five minutes, (ii) that secreted up to half an hour, and (iii) that secreted up to one and a half hours after injection. Sample (i) was always negative on the isolated uterus; samples (ii) and (iii) showed varying degrees of activity according to the condition of the ovary. The rate of flow of the C.S.F. is greater in the first half hour than later; therefore, any secretion of pituitrin will tend to be more concentrated later. The technique for standardising the C.S.F. for pituitrin has been described in detail by Dixon.

Ovarian extracts of both the active and inactive varieties have been added directly to the bath containing the isolated uterus: they all exert a mild stimulant action of the same nature as that produced by most other animal tissues extracted in the same way but considerably less than that of an equal weight of extracted liver. All the ovarian extracts had much the same effect: those which caused secretion of the pituitary did not differ in their action on the uterus from those which were inactive on the pituitary. Liquor folliculi is also without specific uterine action. We have convinced ourselves that these extracts are without any direct specific action on the uterus or on any other form of plain muscle. The following is a record of the experiments with C.S.F. after the injection of the ovarian extracts.

TABLE I. Experiments on rabbits.

Exp.	Weight of ovaries	Result
1. Pregnant 8 days	1.2 grms.	Negative
2. Shortly after parturition	1.85	Positive
3. Two rabbits both 30 days after coition or just before parturition (one making nest)	1.5	"
4. Two rabbits both 31 days after coition or just before parturition	1.0	"

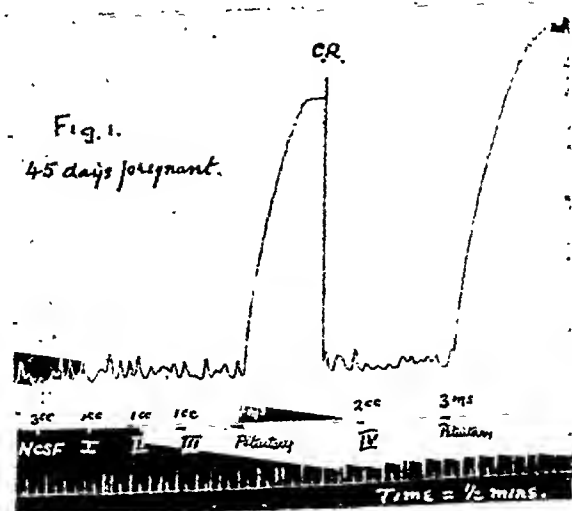
The results with the rabbits, though definite, were not great, owing apparently to the small size of the ovaries and the small amount of extract obtained. It was resolved therefore to employ sows, in which the ovaries are much larger. We were fortunate in being able to avail ourselves of the resources of the University Farm where the complete history of the sows used was known in each case. Our thanks are due to Mr H. R. Davidson, Demonstrator in Agriculture, under whose superintendence the sows were kept. Mr Davidson not only placed all his records at our

disposal but made many observations of his own on the recurrence of œstrus, etc., and the condition of the sows as they approached the time of farrowing. The period of gestation in the sow is usually from 113 to 115 days.

TABLE II. Experiments on sows.

Exp.	Condition	Weight of ovary (or ovaries)	Number of corp. lut.	Result
1.	Pregnant 37 days. One ovary used	6.15 grms.	8	Negative
2.	Pregnant 45 days. One ovary used	9.75	12	"
3.	Pregnant 51 days. (Had only one ovary)	11.65	14	"
4.	Pregnant 62 days	8.75	9	"
5.	"	11.0	11	"
6.	Pregnant 102 days	6.75	6	Slightly positive?
7.	"	8.05	8	Negative
8.	Pregnant 110 days. One ovary used	12.7	11	Positive but not markedly so
9.	Pregnant 114 days	5.5	3	Emphatically positive
10.	Pregnant 114 days. (The sow was preparing to farrow at the time of killing)	7.1	6	" "
11.	Pregnant 115 days	8.15	9	" "
12.	"	3.0	2	" "
13.	Coming on heat. One ovary used	4.8	—	Positive
14.	Due on heat, day after killing. (One ovary used; contained many large follicles as well as old corp. lut.)	6.25	—	Slightly positive
15.	On heat. Both ovaries used. (Ovaries contained 6 or 7 newly ruptured follicles but no old corp. lut.)	4.5	—	Positive
16.	On heat. Ovulation had not yet occurred	3.0	—	"
17.	Two days after heat. Both ovaries	5.5	6	Slightly positive
18.	Six to eight days after heat. Both ovaries	11.2	11	Very slightly positive
19.	Ten days after heat. Both ovaries	17.4	17	Negative

Some of these experiments we give in further detail. No. 2 was a typically negative experiment though all the conditions were favourable



for a positive result. The c.s.f. flowed freely throughout the experiment and the uterus upon which it was tested was sensitive. Fig. 1 is a record of this experiment showing throughout small automatic movements of the uterus. Normal c.s.f. (3 c.c.) as well as the c.s.f. collected up to 5 minutes, 5-30 minutes, 30-60 minutes, 60-90 minutes after the ovarian injection, represented on the tracing by the figures I, II, III, and IV, respectively, are without action on the uterus whilst three drops of 1 p.c. pituitrin added to the bath cause maximal contraction. The bath contained 80 c.c. of Ringer. (c.r. signifies change Ringer.)

No. 8 shows the effect of ovarian extract prepared towards the end of pregnancy, that is, within about four or five days of parturition. The fraction of c.s.f. most active was that secreted 45-90 minutes after injection. Fig. 2 shows the effect of adding 3 c.c. of this fluid to the bath

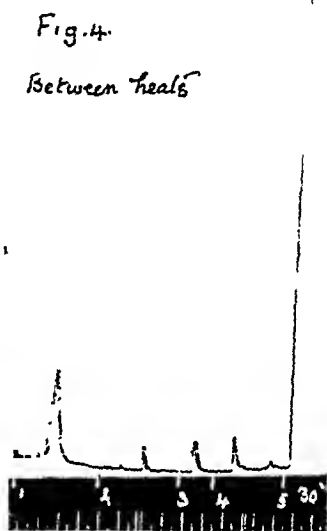
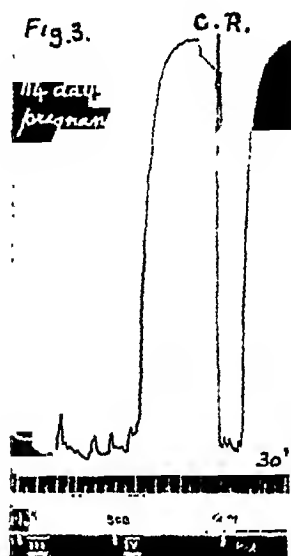


containing the uterus. It will be seen that both the tone and automatic movements increase. The effect is positive though not markedly so. In this experiment 5 c.c. of normal c.s.f. produced no effect.

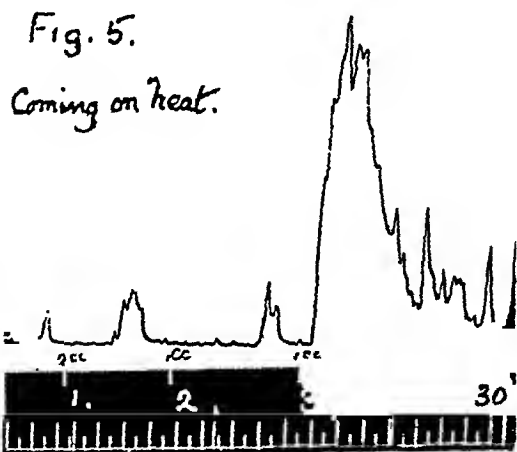
Nos. 9 and 10 were much the most positive of all the experiments. One ovary, weight 7.1 grms. after boiling and filtering, was injected into a male dog weighing 12 kilos. The c.s.f. was secreted abundantly throughout the experiment and Fig. 3 shows the effect of adding 3 c.c. to the Ringer bath. The uterus contracts immediately to a maximum. The Ringer's solution was then changed and after a wait of one-quarter of an hour two drops of 1 p.c. pituitrin added to the bath produced an identical effect. Samples of this c.s.f. produced typical pituitary effects on the blood-pressure of decerebrate cats. The first c.s.f. secreted had no action on the uterus.

No. 19 was a pig which was last on heat on March 1st; it was killed

on March 11th, that is, roughly half-way between heats. Both ovaries were used making a total weight of 17.4 grms.; that is, about three times

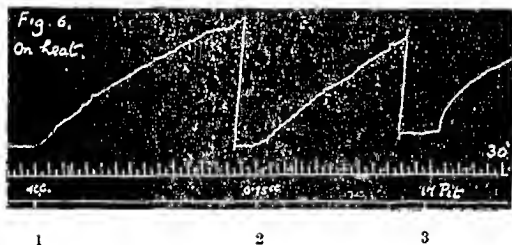


as much ovarian substance was used as was employed in No. 9. The c.s.f. was entirely without action on the guinea-pig uterus. Fig. 4 shows this effect. At 1, 2 c.c. normal c.s.f. added. At 2, 2 c.c. c.s.f. collected up to five minutes. At 3, 2 c.c. c.s.f. collected up to half an hour. At 4, 2 c.c. c.s.f. collected up to an hour and a half; and at 5, 2 drops of 1 p.c. pituitrin which produced maximal contraction.



No. 13 was an ovary obtained from a pig which was coming on heat. One ovary, weight 4.8 grms. was used and after boiling and filtering the macerated ovary with 20 c.c. of Ringer the filtrate was injected into a male dog weighing 11.4 kilos. 2 c.c. of normal c.s.f. and 1 c.c. of c.s.f. obtained immediately after injection were without action on the uterus, but the secretion from 30 minutes onwards was positive. Fig. 5 shows the record in which 1 represents the addition to the bath of normal c.s.f.; 2, c.s.f. secreted in the first 5 minutes; and 3, secreted up to 90 minutes.

No. 15 was a pig killed whilst on heat. The ovaries were small and both were used. The injection was made into a bitch weighing 7.5 kilos. which had been recently on heat. In this instance the normal c.s.f. showed some oxytoxic action. 4 c.c. normal c.s.f. produced an effect about equal to that of one drop of 1 p.c. pituitrin. After the injection, however, the amount of oxytoxic substance was greatly increased since now 0.75 c.c.



c.s.f., collected about one hour after injection, produced an effect corresponding with the 4 c.c. of normal fluid. This is shown in Fig. 6. 1, shows the result of adding 4 c.c. normal c.s.f.; 2, 0.75 c.s.f. collected one hour later; and 3, one drop of 1 p.c. pituitrin. This experiment is unfortunately the only one we have performed on an animal which had recently been on heat and we are therefore unable to state whether the excess of pituitary in the c.s.f. is an accidental occurrence or the result of the condition of the bitch.

One experiment which is not recorded in the table consisted in the injection of 6 c.c. liquor folliculi into the vein of a dog. The c.s.f. secreted in the next two hours was completely negative on the uterus in doses of 2 c.c.

In an earlier paper by one of us it was found that when using commercial extracts of ovary of unknown origin in most cases the

secretion after injecting ovarian extract commenced at once and passed off quickly. In the present experiments, conducted entirely with fresh organs, the whole histories of which were known, we have failed to obtain an active secretion immediately after injection. The secretion of pituitary, though it may begin immediately, is not sufficiently concentrated in the c.s.f. for estimation until perhaps half to one hour after injection. One reason for this may be that the c.s.f. becomes progressively less as the experiment continues so that the secretion would become more concentrated and therefore more readily assayed. The discrepancy, however, is not clear, and we shall return to it again.

It will be realised that at the beginning of the "heat" periods the ovaries contain ripe Graafian follicles which, during œstrus, discharge their ova in the process of ovulation and then become converted into corpora lutea. These bodies become organised in the course of a few days. In the non-occurrence of pregnancy they continue to develop until about mid-way between the heat periods and then commence to undergo regression so that at the approach of a new heat period they are in a state of advanced involution and have, so to speak, made way for the maturation of a new batch of follicles ready for ovulation at the coming œstrus. If, on the other hand, pregnancy supervenes, the corpora lutea continue to undergo hypertrophy until, at or somewhat after, mid-pregnancy and then undergo a gradual involution which, as Corner has shown for the sow, is not completed until shortly after parturition.

The last three experiments recorded above were with ovaries of non-pregnant sows at varying intervals after ovulation and œstrus. After two days (No. 17) the corpora lutea would not have been fully formed, and the result of the experiment was positive. In the next experiment (No. 18) the sow was killed eight days after heat was observed, but it is not known precisely how long the heat may have continued, nor exactly when ovulation took place. It would appear, however, that the corpora lutea were not yet sufficiently developed for the ovaries to produce an entirely negative result when their extract was tested upon the pituitary, for the result recorded is very slightly positive. In the last experiment, in which the sow was killed about mid-way between two heat periods, the corpora lutea spuria had probably reached their maximal development, and the result with the pituitary was negative. In the case of corpora lutea of pregnancy (corp. lut. vera) the organs continue to hypertrophy for a much longer period and reach a correspondingly larger size (Exps. 1 to 8). The entire series of experiments in which ovaries with corpora lutea of different ages were used should be considered in con-

nection with Corner's records (1915, 1921) of the life-history of the corpus luteum in the sow.

Our experiments consistently support the view that in the presence of fully formed and presumably functional corpora lutea the normal ovarian secretion is largely or entirely in abeyance, and this is the condition for a short part of the time between the heat periods, but more particularly during pregnancy. In other words, the corpus luteum may be supposed so to dominate the ovarian metabolism at these periods that the ovarian secretion, which at other times activates the pituitary, is inhibited or else is neutralised by the secretion coming from the corpus luteum. At the close of pregnancy, when the corpora lutea are in an advanced stage of involution, the normal secretory activity is once more produced, and the pituitary is excited to secrete in greater quantity. When the threshold stimulus of the pituitary secretion upon the uterus is reached and passed the pains of labour set in and parturition results. The well-known phenomenon of the growing irritability of the uterus in the latter stages of pregnancy is explained as being functionally correlated with the involution of the corpus luteum. It is to be pointed out that although in our experiments ovarian extract gave positive results at all times excepting when the organs contained well-developed (and so presumably functionally active) corpora lutea, the effects produced were undoubtedly more marked in the case of the ovaries obtained at or about the time of labour (Exps. 9-12), and it may be, therefore, that the hormone which stimulates the pituitary is produced in greatest abundance at these periods. For the purpose of the theory suggested above, however, it is only necessary to assume that this hormone is *not* produced in the presence of functionally active corpora lutea. The marked uterine contractions which are commonly said to occur during menstruation are to be explained similarly, that is to say, they take place when corpora lutea are absent from ovaries which are otherwise very active.

We wish to make it clear that we do not suggest that the ovario-pituitary endocrine mechanism is the sole factor in producing the labour pains. No doubt the foetus itself acts as a direct stimulus, and without the foetus the intense muscular contractions would not occur, but it seems equally clear that the onset of labour cannot easily be accounted for without postulating some further exciting cause apart from the foetus and uterus.

There are certain difficulties to explain if this view be accepted and these may now be briefly considered.

The theory does not account for premature delivery or abortion. In

such cases the stimulus is usually or always extraneous. Abortion may be due to disease, or to drugs, or to shock, fright or physical fatigue, and the stimulus is probably as a rule nervous and not due to pituitary secretion. The conditions are altogether abnormal, and the natural physiological mechanism is not involved. It is known, however, that the mere presence of dead fetuses is not sufficient to bring about delivery (Hammond, 1912).

A more serious difficulty is encountered in the consideration of those cases in which the ovaries have been removed during the latter stages of pregnancy and yet the pregnancy has continued. It is not definitely known, however, that in these cases the duration of pregnancy was for the usual time, or that the course of parturition was normal, and it is not improbable that some compensatory mechanism may have come into existence. It is known that after ovariectomy the pituitary gland undergoes hypertrophy, and although this hypertrophy is generally said to relate to the anterior lobe only, it may be pointed out that there is some evidence of a functional connection between the anterior and the posterior lobes (Blair Bell, 1916). Further, it may be remarked that the normal course of parturition is known to depend upon the integrity of the nervous system, and that there is a presiding centre co-ordinating the various muscular movements in the lumbar part of the spinal cord. Nevertheless, parturition has been known to occur in the absence of the nervous mechanism, as in Simpson's experiment on a sow, and Goltz's on a bitch, in both of which the dorso-lumbar part of the cord was excised, as well as in various cases of paraplegia in women (Brachet, Routh, etc.). So, similarly, it is possible that under some circumstances parturition may occur in the absence of the ovario-pituitary endocrine mechanism, and it is unlikely that a contractile organ like the uterus would retain the foetus indefinitely.

Another difficulty arises from the consideration of the varying periods over which the corpus luteum persists. It is well known that in many animals, whereas the corpus luteum is the same in its earlier stages, it has a more prolonged tenure of existence under conditions of pregnancy, and hence the distinction between the so-called corpus luteum verum and the corpus luteum spurium. It must be pointed out, however, that this distinction does not apply to all mammals, and that although in most species the persistence of the corpus luteum depends upon pregnancy, the organ begins to undergo retrogression some time before the close of pregnancy; that is to say, the presence of the embryo or of the placenta cannot maintain the corpus luteum indefinitely. The manner in which the embryo or placenta acts upon the corpus luteum is unknown, but the

fact that it does influence the duration of the corpus luteum in no way precludes the possibility that the latter organ has an inhibitory influence on the ovario-pituitary endocrine mechanism during pregnancy. It may well be that the conditions of pregnancy are what determine the prolongation of the existence of the corpus luteum verum, and yet that the eventual regression of the corpus luteum is what admits of the ovario-pituitary endocrine mechanism finally asserting itself by exciting the contraction of the powerfully developed uterine muscles.

Attention must also be drawn to the probable bearing of these results upon ovarian and pituitary therapy, especially in relation to parturition. In the preparation of ovarian extracts it is of the utmost importance that account should be taken of the stages of pregnancy, or of the œstrous cycle, of the animals from which the ovaries are obtained for the purpose of manufacturing extracts. It will have been seen that widely divergent results were obtained from ovaries at different periods. Moreover, it is not sufficient merely to discriminate between ovaries which contain corpora lutea and those which do not, since in the closing stages of pregnancy when the corpora lutea are in a condition of advanced involution, they are still of very considerable size, and superficially and macroscopically are very similar to corpora lutea at an earlier stage of pregnancy, when they may be presumed to be in a state of full functional activity. Before utilising ovaries for making extracts it is essential that due account should be taken of the precise condition of the animals from which they are obtained in regard to the occurrence of ovulation, œstrus and pregnancy.

SUMMARY.

1. The phenomena attending the termination of pseudo-pregnancy are cited as pointing to the conclusion that the occurrence of a certain stage in the cyclic activity of the ovary is a factor in parturition.
2. There is no evidence that the ovarian secretion brings about parturition by acting directly on the muscles of the uterus.
3. The secretion of the pituitary has a definite specific action on the muscle of the uterus.
4. The secretion of the ovary in the absence of fully formed (and therefore presumably functional) corpora lutea has a definite specific stimulating effect in promoting pituitary secretion.
5. The secretion of the ovary in the presence of such corpora lutea, on the other hand, has no such effect, its action on the pituitary gland being completely negative.

6. The corpus luteum so dominates the ovarian metabolism during pregnancy as to inhibit the normal ovarian secretion, but with the involution of the corpus luteum at the close of pregnancy the uterus shows an increasing irritability, and eventually, when the normal ovarian endocrine mechanism is sufficiently re-established, the secretion, working through the intermediation of the pituitary, becomes an important factor in bringing about labour.

7. The action of the ovary upon the pituitary undergoes a similar variation in the absence of pregnancy and during the oestrous cycle, according to whether corpora lutea are present or absent.

8. The bearing of these experiments upon ovarian and pituitary therapy, especially in relation to parturition, is briefly referred to.

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THE INFLUENCE OF INSULIN ON THE BLOOD SUGAR IN THE HEART-LUNG PREPARATION.

By F. PLATTNER (*Fellow of the Rockefeller Foundation*).

(*From the Institute of Physiology, University College, London*)

ONE of the main results of the previously published investigations dealing with the problem of the site of action of insulin is the observation that the surviving mammalian heart causes an increase in the normal rate of disappearance of glucose from the perfusing fluid when working under insulin. Hepburn and Latchford⁽¹⁾ found the average sugar consumption of the isolated rabbit's heart perfused without insulin to be 0.87 mgm., while in experiments with insulin the average consumption increased to 3.06 mgm. per gram of heart per hour. Burn and Dale⁽²⁾ also found a marked increase in the sugar disappearance from the perfusion fluid due to insulin. In all these experiments, however, the perfusion was carried out in the apparatus described by Locke and Rosenheim⁽³⁾ with oxygenated Locke's solution (Hepburn and Latchford, Burn and Dale), or with blood diluted with an equal volume of Locke's solution (Burn and Dale), and in the latter case the increase was not found to be as high as in the former one. As the normal conditions under which the heart does its work are not imitated in a satisfactory manner in this method it seemed to be of some importance to check the results obtained in the above mentioned way by examining the influence of insulin on the blood sugar in a heart working under as nearly normal conditions as possible. In Starling's heart-lung preparation⁽⁴⁾ we possess a method in which these conditions are fulfilled and it is with the results obtained by adding insulin to the heart-lung preparation that the present paper is concerned.

Methods. The experiments were carried out on dogs. After the preparation was ready usually about 1 gm. of glucose¹ was added to the blood and was allowed to circulate for 15 minutes before the first sample of blood for sugar estimation was taken. Further samples for sugar estimation were taken every half hour, in some experiments every 15 minutes. After the end of the first hour (immediately after the third or

¹ It may be mentioned that two experiments showing the same result were carried out without any addition of glucose.

6. The corpus luteum so dominates the ovarian metabolism during pregnancy as to inhibit the normal ovarian secretion, but with the involution of the corpus luteum at the close of pregnancy the uterus shows an increasing irritability, and eventually, when the normal ovarian endocrine mechanism is sufficiently re-established, the secretion, working through the intermediation of the pituitary, becomes an important factor in bringing about labour.

7. The action of the ovary upon the pituitary undergoes a similar variation in the absence of pregnancy and during the oestrous cycle, according to whether corpora lutea are present or absent.

8. The bearing of these experiments upon ovarian and pituitary therapy, especially in relation to parturition, is briefly referred to.

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THE FORMATION OF THE *v* WAVE IN THE VENOUS PULSE. By D. T. BARRY.

(From l'Hôpital Lariboisière, Paris, and University College, Cork.)

IN simultaneous venous and carotid records the dicrotic notch of the latter may precede the tip of the *v* wave in the former by about .08 sec. to .12 sec. The difference between transmission time in carotid and jugular is so slight that the two points indicated would seem to correspond to events in the cardiac cycle which are separated from each other by about this interval of time. These events are: the closure of the semi-lunar valves which occasions the dicrotic notch, and the opening of the a.-v. valves which is supposed to occasion the decline of the *v* wave. It would therefore scarcely be correct to state, as some writers do, that the end of systole is marked by the dicrotic notch in the arterial and by the apex of the *v* wave in the venous pulse, as if these two things occurred simultaneously. The position of the apex of *v* varies slightly, but it is shown in sensitive optical records to be almost coincident with the dicrotic notch in the carotid. Some say that *v* begins before the end of systole, while others say it begins when the semi-lunar valves close. Much depends on what is to be considered as the beginning of the wave. The venous rise in pressure begins before systole is ended, but the end of this systolic rise (due to systole of the ventricle) is not necessarily the same as the top of the *v* wave; some hold that this latter is a diastolic event as we shall see in a moment. In ordinary arterial curves taken from the femoral or from the radial the dicrotic wave is often seen corresponding to the apex of *v* in the jugular owing to delay in transmission of the pulse to the distal artery, the venous event being later at the source than the arterial. But optical records in these conditions may show the tip of the *v* wave preceding the dicrotic. The interval between the two events at the source is sometimes referred to as the isometric period of diastole, thus connoting the formation of the tip of *v* as a diastolic occurrence. The interval is occupied by the second sound of the heart which begins with closure of the semi-lunar valves and is continued over the shoulder of *v*.

The *v* wave is most commonly attributed to a rise in venous pressure or a holding up of the venous current during ventricular systole, the

sudden release afforded by the opening of the auriculo-ventricular valves causing a fairly sharp decline of this pressure; hence it is called a stasis wave. There is little doubt that this process can explain the gradual slope of the curve upward and its decline, but it does not explain the very sharply formed top which is sometimes seen on the wave especially in sensitive optical curves. With ordinary levers one occasionally sees vibrations, two or three in number, or a mere doubling, on the summit of v , of which vibrations one is higher than the others and forms the

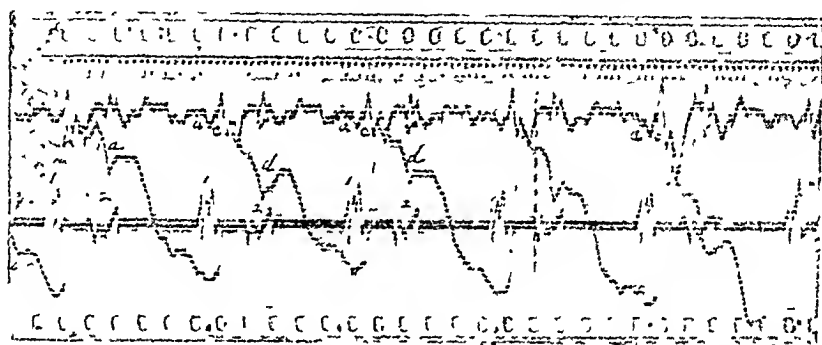


Fig. 1. Venous (top), sound (middle) and arterial (carotid) curves. Vibrations not shown on the dicrotic d . Note last sharp v wave. The frequency of 1st sound is about 40. Time 0.05 sec.

apex. In optical records this apical vibration is often very sharp and tall (Figs. 1 and 3) and coincides closely with the dicrotic notch, d of the carotid, coming about the centre of the second sound. In triple records—jugular, carotid, heart sounds—a series of vibrations corresponding to those of the second sound is seen on the dicrotic wave as well as on the upstroke of the v wave, and careful counts of the frequency of these show that all three sets are at practically the same rate (Fig. 2). The tambours employed in taking the records possessed a vibration frequency of 50 to 70 per second. They have been sometimes interchanged for the different curves but with very little differences in the appearances of the records. Physicists say that in tambours of the kind used, over-tones may occur from either crucial or circular nodes on the membrane at from 2.3 to 3.6 times the fundamental rate. Where pulses impinge on the centre of the membrane, as happens with the instruments employed, the circular node is the only one likely to occur, giving accessory waves of more than three times the fundamental number. This makes it unlikely that they could cause confusion in counting.

Given three sets of vibrations of similar frequency occurring syn-

chronously on the records one naturally seeks a common cause for all three. The second sound of the heart is well known to be due to the

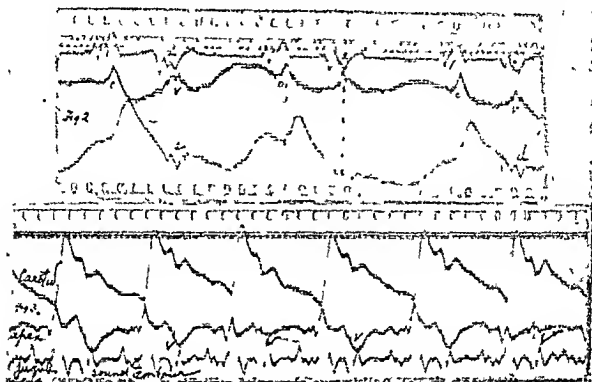


Fig. 2. Sounds (top), jugular, carotid (bottom). The sounds in this figure are crude. The speed of paper nearly twice that of Fig. 1.

Fig. 3. Venous pulso tracing (bottom) with sound tambour cutting out effects of stasis. Note undulations at position of v wave.

vibrations of the semi-lunar valves. The frequency varies in different individuals, and its pitch, both absolutely and relatively to that of the first sound, is variously estimated by different observers; the discrepancies in the figures given may with advantage be considered briefly. Gerhartz(4) allows a margin of 34 to 74 for the first sound and 35 to 82 for the second, with an average of 39.4 for the first and 47.5 for the second. Einthoven(2) gives over 80 for the first and 45 for the second. Weiss and Joachim(9) find over 90 for the first and 80 for the second. In the records taken by the optical method which are at present under consideration the average frequency for the first sound in a large number of investigations was 40 to 50 and for the second 45 to 55. The maximum registered for the second sound was 70 (14 vibrations in .2 sec.). The total number generally seen varied from 3 to 9 for both sounds. The adjustment of the opening in the T-piece connected with the tubing of the sound tambour for cutting out the apex beat is of great importance in procuring good records. By careful manipulation a good sensitive combination can be arrived at which is little inferior to a soap or rubber

solution film such as that used by Weiss and by Wiggers. In that way some reliance may be placed in the method as a crude one for investigation of the sounds in themselves, but it is especially as a means of signalling the positions of them and thus showing the onset of systole and diastole that it is useful. The rubber used should be very thin and possess a high coefficient of elasticity for accurate records; for mere signalling the ordinary rubber of a finger stall does quite well. Strips of silvered cover-glass about 1 cm. long and 1 mm. wide serve as reflecting mirrors. They must be extremely light so as to diminish the vibrating mass as much as possible.

The first sound usually begins with an upward swing, that is, with a slightly increased pressure within the tambour (Fig. 1). When recorded together with the apex beat (cardiogram) the sound vibrations precede the stroke of the latter, and on the cardiogram itself when taken by a very sensitive tambour there is an initial swing or two corresponding with the beginning of the sound. The second sound begins with a downward or negative swing and the subsequent vibrations gradually mount above the neutral line. When the side opening of the sound tambour is almost closed and the position of the receiver on the chest is favourable—to the right of the apex beat—there may be a sharp downward stroke at the beginning of systole and a sharp upstroke at diastole due to increased intra-thoracic pressure, the latter coinciding very closely with the *v* wave (Fig. 4). The picture is different from that of an inverted

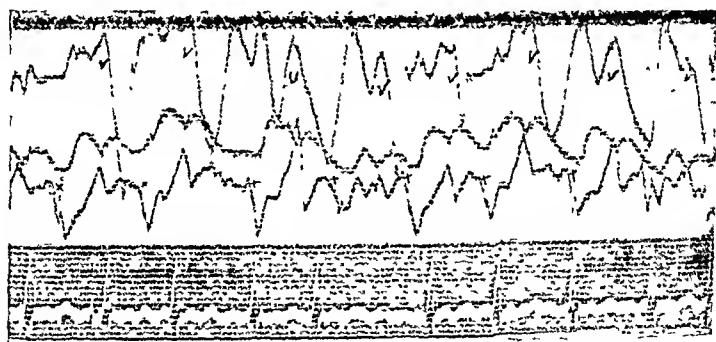


Fig. 4. Venous (top), arterial and apex (bottom) curves from a case of auricular fibrillation. Electrocardiogram added, not simultaneous, but taken a few minutes after optogram and at same speed of paper. Beginning of systole in apex curve marked *x*.

cardiogram. It is a useful procedure to adopt in cases of irregular rhythm on account of the marked downward and upward deflections, the latter

pointing at once to the *v* wave, as in Fig. 4, from a case of auricular fibrillation under treatment.

It seems to me that the top of the *v* wave in the jugular pulse is not simply the end of a period of stasis but has to be accounted for in some other way; vibrations are seen on it corresponding to those of the semi-lunar valves, whether effected through the ventricle and a.-v. valves or along the arterial walls I cannot say. In favour of auricular conduction is the fact that in many curves taken from the right auricle or the superior vena cava in the dog a series of vibrations corresponding in time and rate to the second sound are found. I have several of these taken from the heart-lung preparation. Frank^(see 4) gives a vibration frequency for the second sound in the dog of about 45, which corresponds to the rate in this record, the *v* wave or its equivalent phase showing four to five vibrations in one-tenth of a second. The chest of the animal was opened, a fact which alters the usual form of the venous record. The frequency of these vibrations sometimes changes, as well as the amplitude, and near the top of the wave some other agency comes in causing one or two swings of slower rhythm which give a well marked outstanding summit to the *v* wave. What is this agency? The position on the *v* wave where vibrations *begin* varies somewhat; occasionally the first seen, even the only one, is that forming the apex, and where this occurs there is generally no doubt about the change in speed of the venous rise before the apex is reached, a change from a gradual slope to a sharp ascent. The swing is almost synchronous with the dicrotic notch in the carotid but it occurs at a distinct interval of nearly one-tenth of a second after the beginning of the second sound, and is sometimes succeeded by a rounded shoulder which marks the opening of the a.-v. valves.

We have thus two things happening about the moment that the summit of *v* is to be formed, namely, the swing of the semi-lunar valves which undoubtedly affects the venous pulse and produces corresponding vibrations on it, and an increase in intra-thoracic pressure at the beginning of diastole. Either or both of these agencies can produce a quick rise in venous pressure and give a sharp summit to the *v* wave and the picture shown of this wave is now of one effect and now of both. It is diastolic in time if we take the second sound as marking the beginning of diastole.

There are other waves in an intra-auricular record which are occasioned by secondary vibrations of the tricuspid valve, and corresponding to these we often see the *b* wave, described by Gibson⁽⁵⁾ and by Hirschfelder⁽⁶⁾, in the jugular pulse with which a third sound

may be inscribed. Is it possible that this valve would also vibrate in a similar way about the period of opening? If so it is very difficult to imagine how the necessary upward swing to cause the v summit could be effected. The tricuspid valve accordingly cannot be regarded as the agency when considered in this way. But some observers consider that tricuspid regurgitation may be a factor in the formation of v . Mackenzie(8) mentions three possible ways in which this venous rise may be formed: by stasis, by tricuspid regurgitation and, according to Gerhardt and Wenckebach(3) whom he quotes, by *push* on the a.-v. valves after closure of the semi-lunars—a diastolic origin. What could cause such a push unless closure of the semi-lunars? When regurgitation takes part in it the summit is early, but it must be remembered that regurgitation is not confined to systole; it persists for a fraction of a second in diastole, as is shown in the curves of Wiggers and Feil(10), and in curves obtained by myself(1) in experimental insufficiency in the dog. Tricuspid regurgitation is common according to Mackenzie; it is easily provoked and it may exist in practically normal conditions. Difficulties of output in the left side of the heart readily lead to it.

Any or all of the factors mentioned may play a part in the formation of v , but so far as the sharp top of the wave goes none of them, unless it be the mechanism described by Gerhardt and Wenckebach, is sufficient to account for it. I have at present no access to the original papers of these authors but the account of Lewis(7) who interprets their observation as a rebound at the a.-v. ring on cessation of systole, is that adopted for application to the question now at issue. The main rise of venous pressure from the dip, x' , may be accounted for by stasis during systole, but there is super-imposed on this a diastolic factor which forms the sharp top, and this top shows very clearly in sensitive curves, though the extent of the v wave formed in this way may be sometimes small.

It seems to me that the intra-thoracic pressure-changes during the cardiac cycle have not been fully investigated so far as their effects on the venous pulse go. A sensitive sound tambour used for optically recording can be so adjusted as to give a sharp downward movement of the beam for the first sound, that is the beginning of systole, like an inverted cardiogram (Fig. 4) and a sharp upstroke at the beginning of diastole, when the receiving tambour is to the right of the apex. This sharp diastolic upstroke corresponds very closely to the v wave of the venous pulse taken simultaneously, so much so, that one asks if the summit of v or the part of this wave which is distinctly shown in optograms to be diastolic in time, may not be due to an intra-thoracic pressure-

change occurring at the beginning of diastole. When the heart swells in diastole there is a sudden increase of pressure in the thorax which causes a positive reflex wave in the veins. This comes in near the top of the passive rise due to systole and changes what would otherwise be a slowly formed shoulder to a sharp peak, the *v* wave. With or without this diastolic pressure effect the vibrations of the semi-lunar valves affect the venous pulse, as a perforated tambour system, which is practically unaffected by static effects, can show (Fig. 3).

Conclusion. A sharp apex to the *v* wave is shown by sensitive tambours to be formed by some rapidly acting force before the a.-v. valves open. One factor in this is certainly that of swing of the semi-lunar cusps; a second is probably the rise in intra-thoracic pressure in diastole.

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HEAT REGULATION AND WATER EXCHANGE.
V. The Phase of Blood Dilution in Man. BY H. G. BARBOUR,
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THERE is a considerable range of environmental conditions in which it is of high importance for the body to increase its heat loss by radiation and related factors. When, on the other hand, the body becomes exposed to a temperature above that of its own, radiation works in an adverse direction and recourse must be had to evaporation.

That excessive heat increases the concentration of the blood has been clearly recognised, especially since the work of Haldane⁽¹⁾, but only in recent years has it been pointed out that the water content of the blood is of high significance in connection with radiation. Barbour and Hermann⁽²⁾ showed blood dilution to be an advantageous accompaniment of antipyretic drug action, and the first-mentioned author⁽³⁾ demonstrated a decrease in the blood solids of dogs exposed to a 40° C. neck bath allowed to cool gradually, and Barbour and Tolstoi⁽⁴⁾ described experiments upon dogs in which a majority of 42° C. baths increased its concentration. The matter has recently been further clarified by the experiments of Lozinsky⁽⁵⁾ who placed dogs in a constant temperature hot chamber under both moist and dry conditions. A definite dilution of the blood was always found at the highest environmental temperatures which, under the given condition of humidity, could be tolerated without an increase in body temperature. Concentration of the blood and marked panting set in just above the point where the regulation breaks and the body temperature becomes increased.

The blood dilution shows that to promote increased flow through the skin the peripheral blood stream bed can, because of the increased blood volume, be widened without necessarily calling upon the internal organs for a compensatory vaso-constriction. The dilution phase is significant therefore in respect to the increased blood volume, favouring radiation, as well as to the mobilisation of water for sweating and heat polypnea.

Dilution of the blood with marked exertion has been observed by Kostner(6) and his collaborators who showed that a protein-salt solution streamed into the blood under this condition. Cohn(7) and Eckert(8) found some evidence of a similar dilution of the blood under the influence of electric light baths. In the tropics Young, Breinl, Harris and Osborne(9) found the blood sometimes diluted and sometimes concentrated under moderately warm conditions; but recently Barcroft and others(10) have given a clear demonstration of the dilution phase in man, as a result of observations incidental to the work of the Peru High Altitude Expedition. Barcroft, Meakins, Davies, Scott and Fetter(11) found that high blood volume determinations (Haldane method) obtained in the tropical seas were best accounted for by environmental temperature conditions. They have reported two experiments carried out in a glass respiration chamber in the Cambridge Physiological Laboratory, in which the subjects were kept for two and three days respectively at a temperature of from 32° to 35° C. They observed first the phase of dilution of the blood, shown by reduction in the hæmoglobin content, accompanied by an increased blood volume without change in the total oxygen capacity. After some hours adjustment in the hæmoglobin content was secured, not apparently by a return to normal conditions but by an increase in the absolute number of the red blood corpuscles, thus increasing the total volume. In other words, exposure for two or three days to such a degree of overheating appears to have augmented the whole blood (favourable to radiation) first, as Barbour and his co-workers have described, by increasing the fluid content and later by adding extra red cells. Erythropoietic stimulation by exposure to tropical conditions is also described by Knipping(12).

After it had become apparent from the experiments of Lozinsky that the phase of dilution of the blood in dogs was not fortuitous but could be induced when conditions of humidity and air movement were given as careful attention as the temperature, it was determined to study the phase of blood dilution in man. And it was felt that the observations should be correlated with the environmental cooling-power as a whole rather than with any single factor. This was done by employing Hill's(13) kata-thermometer. From eleven of the warm chamber experiments thus performed we herewith present detailed observations upon the blood.

Procedure. From the veins of the forearm were taken blood samples upon which were made determinations of total solids, hæmoglobin and

amount of variation in the blood from this normal is expressed by the ordinates. A 1 p.c. change in blood solids of course corresponds roughly to a 5 p.c. variation in the hæmoglobin.

From a study of Fig. 1 it becomes evident that the dilution phase of the blood upon half-hour exposures is best exhibited when the subject is exposed to wet-kata readings of about nine millicalories. The depression of the hæmoglobin (O O) at this level amounts approximately to 10 p.c. while the lowest level attained by the total solids (X X) corresponds scarcely to one-third of this amount.

The hæmoglobin changes, confirmed by the oxygen capacity variations, give ample evidence of dilution of the blood, whilst the very slight depression of the total solids indicates that the new fluid entering the blood stream is sufficiently concentrated to keep the blood concentration almost up to its normal level.

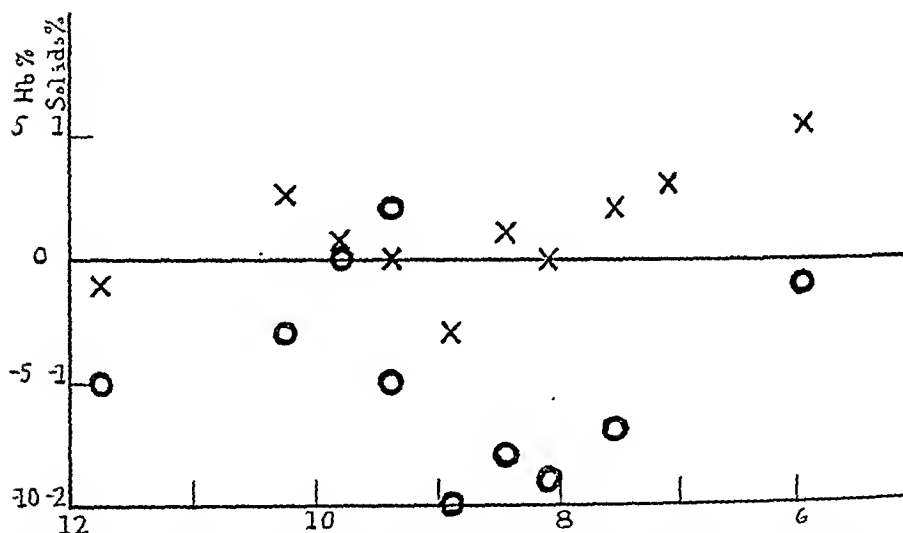


Fig. 2. Same as Fig. 1; but exposures lasted one hour. (One experiment of 75 minutes is shown, at 8.45 millicalories.)

As the experiments progressed through the second half-hour period it will be seen from Fig. 2 that the compensation in the blood solids was usually complete at the end of the first hour; on the other hand, the general level of the hæmoglobin is then at least as low or lower than after the first half-hour. This is especially true of conditions where the wet-kata readings are low (seven to nine millicalories). Thus the need for augmented radiation by increasing the blood volume as well as the need for mobilisation of water for the sweating which, under these

severer conditions, is well under way during the second half-hour, is met by a high degree of blood dilution.

It will be further seen from the two figures that for normal resting man twelve millicalories wet-kata cooling-power is about as low as can be tolerated without blood dilution, while at six millicalories (the point at which the body temperature can no longer be kept constant) conditions not only become too extremo for blood dilution to be of much value but also the point is reached at which inflow of lymph into the blood stream no longer keeps pace with drafts of fluid by the sweating mechanism. This is apparent from the 1 p.o. increase in blood solids noted at the end of the first hour.

CONCLUSIONS.

1. The phase of dilution of the blood found previously in dogs exposed to warm environmental conditions has been clearly demonstrated in man.

2. The optimum condition for the exhibition of the phase of blood dilution in man is a wet-kata cooling-power of eight or nine millicalories per sq. cm. per second.

3. Upon exposure of the subject to heat, the blood evidently becomes diluted by plasma of as nearly as high a total solid content as the normal.

4. The phase of dilution does not occur where the cooling power is above twelve millicalories nor does it persist more than an hour if below six millicalories. At the latter wet-kata reading sweating becomes profuse, the blood concentration increases and the body can no longer maintain its original temperature.

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HEAT REGULATION AND WATER EXCHANGE.
VI. The Mobilisation of Water and Salt in Warm Environments.
By W. F. HAMILTON, H. G. BARBOUR AND N. M. LOOMIS.

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University of Louisville.)*

It has been shown by Barbour and others(1) that a moderately warm environment affects the water content of the blood in man in a manner similar to that previously described for dogs. Exposures of one-half hour to a "wet-kata" cooling-power of eight or nine millicalories per sq. cm. per second produced a slight fall in the blood solids. Over a wider range (about eleven to seven millicalories) a depression appears in the hæmoglobin content which is more marked than that in the solids, and at its peak (eight or nine millicalories) indicates a 10 p.c. dilution of the blood. When like exposures are continued for an hour the blood solids may become so readjusted as to mask the dilution, but the hæmoglobin remains at the same low level as the end of the first half-hour. Thus the theory that dilution of the blood is constantly associated with the regulation of the body temperature against moderate depression of the environmental cooling-power has been definitely confirmed. The relatively slight decrease in the blood solids was attributed to the inflow of a plasma about as concentrated as the normal blood.

The present study was undertaken to follow in further detail the effects of warm environments and especially to secure additional evidence concerning the concentration and composition of the blood from determinations of its specific gravity. This has been done in both men and dogs, proceeding as detailed in the paper mentioned above(1). Specific gravity was determined by the new falling-drop method of Barbour and Hamilton(2).

Results in man. Three normal fasting men were exposed to a wet-kata cooling-power of seven or eight millicalories. Two of the experiments were continued by reducing the cooling-power to a much more severe level, involving intensive sweating. The results exhibited in all three subjects the same dilution of the blood shown in the paper by Barbour and others(1). Marked depression of the hæmoglobin occurred with a

lesser diminution in the blood solids (practically lacking in subject *B*). In the case of *L* (Fig. 1), an approximately constant cooling-power was

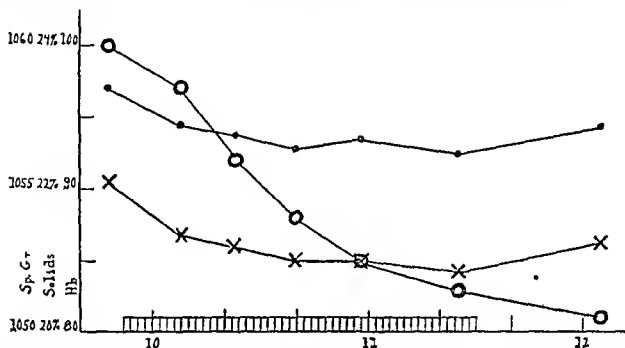


Fig. 1 Subject *L* (●) specific gravity; (X) solids; (O) hæmoglobin (▨) in warm room, wet kata cooling-power of about eight millicalories. Ordinates represent essentially equivalent changes in specific gravity and solids; abscissæ represent time.

maintained throughout the one and one-half hour stay in the warm chamber. During the latter part of the time some adjustment was seen in the specific gravity and blood solids, both of which fell much more gradually than the hæmoglobin. Furthermore they exhibited a partial return to normal one-half hour after the warm chamber was left.

In the other two experiments (Figs. 2 and 3), as soon as some adaptation to the warm environment had set in, the cooling-power was reduced to a much lower level by the introduction of steam into the chamber. Following this was seen in both subjects a new reduction in the specific gravity, while in *H* the solids also were depressed for a second time. These results are attributed to a repetition of the dilution stimulus (sudden increase in severity of conditions). Later, as sweating progressed, re-concentration of the blood occurred but the experiments were discontinued at about the time the normal level was attained. The relation between the specific gravity and solids, which in general pursue a fairly parallel course, will be discussed below, after presentation of the material relating to the dogs.

Results in dogs. From among the experiments upon dogs performed in connection with the present study, four will be considered here. The environmental cooling-power of the warm room was kept in the neigh-

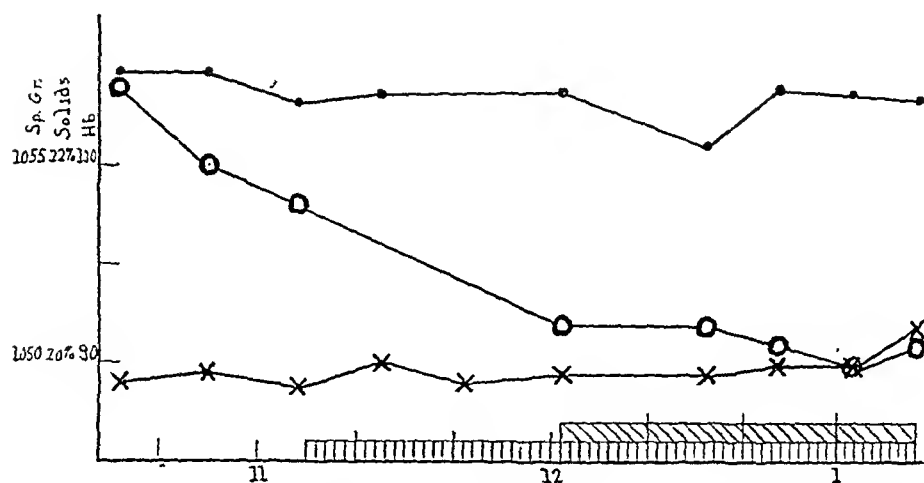


Fig. 2. Subject B. (●) specific gravity; (X) solids; (O) haemoglobin. (▨) in warm room; (▩) wet-kata cooling-power reduced to a much lower level, from about five millicalories down. Ordinates represent essentially equivalent changes in specific gravity and solids; abscissæ represent time.

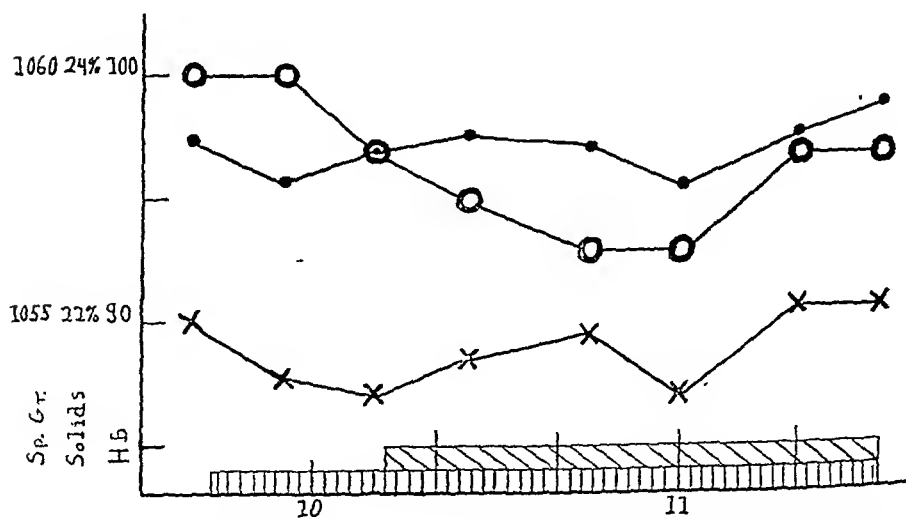


Fig. 3. Subject H. (●) specific gravity; (X) solids; (O) haemoglobin. (▨) in warm room; (▩) wet-kata cooling-power reduced to a much lower level, from about five millicalories down. Ordinates represent essentially equivalent changes in specific gravity and solids; abscissæ represent time.

bourhood of eight to ten millicalories per sq om per second Steam was used and the dry-bulb reading was about 35° C The effects upon splanchnotomised dogs of exposure to such conditions are presented in Fig 4, while Fig 5 illustrates the results of similar treatment of two normal dogs

The *splanchnotomised dogs* were both healthy and showed no effects of double splanchnotomy beyond the slight anæmia indicated by blood solids values below 18 p c in each case An hour's stay in a moderately warm environment in both animals reduced both the blood solids and

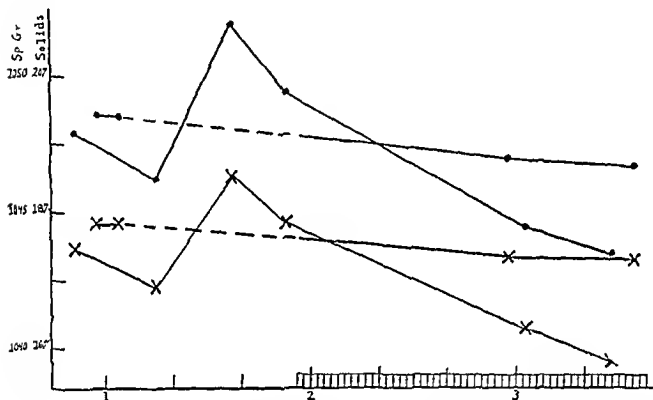


Fig 4 (●) specific gravity, (X) solids, of blood of two splanchnotomised dogs (shaded) warm room exposure First two points on each curve represent findings at normal room temperature The increased concentration shown in one animal is due to an intervening water bath at 29° C Ordinates represent essentially equivalent changes in specific gravity and solids, abscissæ represent time

specific gravity below the original level The preliminary increase in concentration in one dog was due to an intervening water bath of 29° C That the effect of heat is one of simple dilution of the blood without significant alteration in the $\frac{\text{protein}}{\text{salt}}$ ratio is apparent from the close parallelism between blood solids and specific gravity, for two of us (2) have pointed out that the ratio of solids to specific gravity varies in essentially the same fashion as the $\frac{\text{protein}}{\text{salt}}$ ratio

Turning now to exposures of *normal dogs* to a similar warm

environment one sees in Fig. 5 (from two dogs, one of which was first given a 20° C. water bath increasing the blood concentration as shown) that the parallelism between specific gravity and blood solids no longer obtains. When either of these animals had been in the warm room for one hour not only was a blood dilution indicated by both the specific gravity and solids values, but it was also evident that, of the two, the

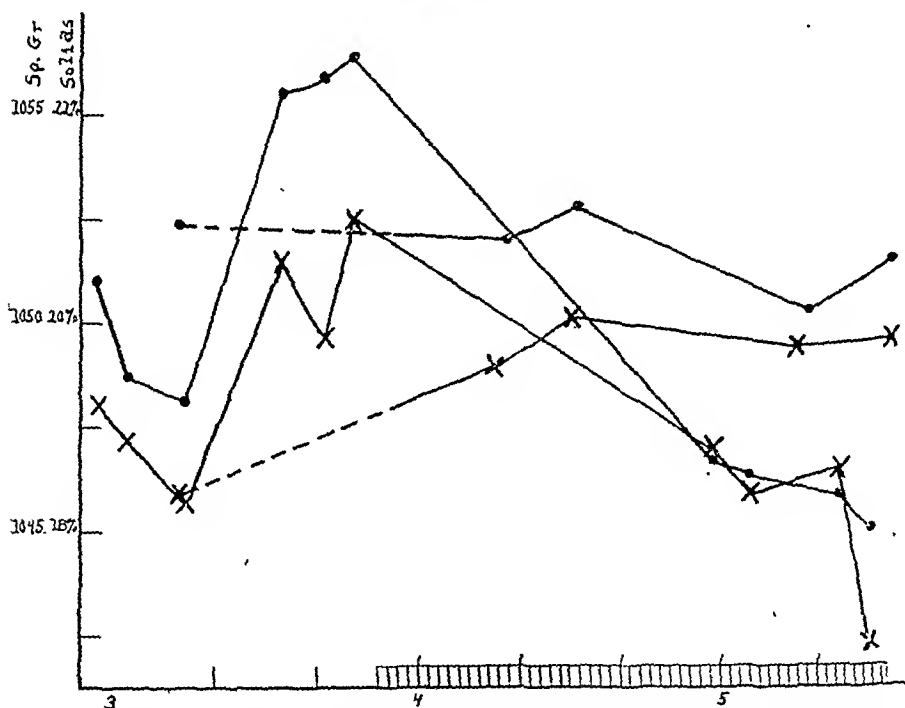


Fig. 5. (●) specific gravity; (X) solids, of blood of two normal dogs. (▨) warm room exposure. First three points represent the normal findings at normal room temperature. The increased concentration shown in one animal is due to an intervening water bath at 20° C. Ordinates represent essentially equivalent changes in specific gravity and solids; abscissae represent time.

specific gravity showed much the greater depression. This can have no other meaning than that the response to certain degrees of environmental warmth in normal dogs involves a reduction in the $\frac{\text{protein}}{\text{salt}}$ ratio. These animals were both panting vigorously at the time of the full exhibition of this effect although their blood had not yet begun to concentrate. Whether the phenomenon is to be attributed to merely a loss of salt from the blood through salivary secretion or also to the stimulation of a mechanism by which some protein-rich fluid is brought into the blood (splanchnic lymph?) will not at present be discussed.

The protein-salt ratio in man. Referring back to the two human experiments in which marked sweating was induced, one may detect there also a tendency towards convergence of the specific gravity and blood solids curves as the end of both of the experiments is approached. The abundant salt-laden perspiration may well account for this rise in the $\frac{\text{protein}}{\text{salt}}$ ratio of the blood. The ratio possibly attains its maximum after about one-half hour of severe sweating, when, as Adolph(3) has shown, the chloride excretion first gets fully under way.

During the phase of blood dilution, on the other hand, one sees some indication that the $\frac{\text{protein}}{\text{salt}}$ ratio may fall. The entire experiment with *L* bears this out and it also appeared with *H* before sweating got under way. *B* may have failed to show it on account of exhibiting a permanently low $\frac{\text{protein}}{\text{salt}}$ ratio. The fall in the $\frac{\text{protein}}{\text{salt}}$ ratio during blood dilution indicates a mobilisation of salt as well as of water.

CONCLUSIONS.

1. Moderately warm environments (wet-kata cooling-power about eight millicalories) induce a fall in the specific gravity of the blood of man and dogs which is of a similar order to the decrease seen in the blood solids and much less than that observed in the hæmoglobin.

2. From the relation between specific gravity and blood solids, evidence is adduced that besides the water mobilised under these conditions there may sometimes be an increase in the amount of salt. The blood, however, is ultimately left with a relatively higher $\frac{\text{protein}}{\text{salt}}$ ratio. This increased ratio is especially marked in normal dogs. In man it may be due to a loss of salt with the perspiration.

3. The $\frac{\text{protein}}{\text{salt}}$ ratio was found scarcely, if at all, increased in splanchnotomised dogs although the dilution of the blood is very evident.

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THE MECHANISM OF THE SPLENIC REACTION TO
GENERAL CO POISONING. BY S. DE BOER (*Fellow of the
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(*From the Physiological Laboratory, Cambridge.*)

IN 1923 J. Barcroft and H. Barcroft⁽¹⁾ showed that when the hæmoglobin of the spleen pulp, in rats breathing .06 p.c. to 1 p.c. CO, is compared with that of the general circulation, there is a lag between the percentage of the COHb in the general circulation and that in the spleen pulp which may attain 30 minutes. They found that a similar lag may attain 90 minutes in rats which had been breathing CO and were exhaling this gas. Heger⁽²⁾ had previously shown that if a dog be poisoned with CO and blood is taken from the various organs as soon as the animal falls into convulsions, the blood of the spleen and the bone marrow retains its normal spectroscopic appearance while that of the general circulation contains a considerable amount of COHb. A similar condition of affairs is found if the animal is poisoned with morphine.

In this research an attempt has been made to throw light upon the mechanism whereby these differences in the COHb content of the hæmoglobin of the splenic pulp and the arterial blood are brought about.

Schafer and Moore⁽³⁾ showed that under the influence of any kind of asphyxia the spleen contracted in the intact animal. However, in the case of CO-anoxæmia it is not perfectly clear that we are dealing with a pure oxygen want or with a pure asphyxia since various observers have found some evidence of a specific action of CO on various tissues. However, many workers have failed to find anything in the nature of the action of CO on any tissue which was not perfectly accountable on the view that the poisonous properties of CO were due to its ability to produce a severe oxygen want; and in view of these researches we have also investigated whether the action of CO on the spleen is due to a specific action of CO on the splenic tissue or to oxygen want or to asphyxia or to a combination of all these causes.

Experiments on the intact animal.

Method. Cats were used throughout the research. No difference in result was found whether they were male or pregnant or non-pregnant

females. We had some difficulty in finding a suitable anæsthetic. Chloroform, ether and alcohol mixtures did not give a steady enough anæsthesia for long experiments. Urethane appears to have an inhibitory action on the splenic muscle, and paraldehyde, while giving a very steady anæsthesia, has a disturbing effect on the vagal action on the heart and on various vaso-motor reflexes so that it is impossible to perform the necessary control experiments in which the effect of an independent fall or rise in B.P. on the spleen is investigated. The most suitable anæsthetic is luminal(4) which we sometimes gave to the cats in their food in the form of the free acid, and sometimes injected subcutaneously in the form of the sodium salt. The dose was 0.2 gm. per kilo body weight as recommended by Sykes. The animals become almost poikilothermic under luminal and to obviate the difficulties arising from temperature changes the cat was placed on an electrically heated table and in the majority of the experiments an electric thermostat was placed in the cat's rectum and by this means a constant rectal temperature of 37° C. or 38° C. was maintained. A glass topped cover was placed over the animal during the experiment.

When anæsthesia was complete, tracheotomy was performed, a cannula tied in the left carotid, and if necessary one in the right jugular vein. The spleen was exposed by a longitudinal incision in the left nipple line. In animals where the tail (anterior end) of the spleen was held so close to the stomach by blood vessels that it was difficult to place the organ within a plethysmograph without injury, or leakage from the plethysmograph, these vessels were doubly tied and cut. This was seldom necessary and in no case were the main vessels of the spleen or their branches to the spleen tied. The plethysmograph used was of the pattern described by Schaffer and Moore(3) which we find to be more satisfactory than the patterns described by Roy(5), Hoskins and Lee-Gunning(6), or by Jackson(7). For recording, an ordinary Marcy-Chauveau tambour was used.

When the experiments were carried out under spontaneous respiration a valve of the pattern described by Mellanby(8) was used. The valve was connected by a two-way tap to a Douglas bag containing a mixture of air and CO of known composition. If necessary a bag of oxygen could be connected. The bags themselves were shown to have no effect of physiological importance on the gas they contained by a series of control experiments in which air was administered to the cat from the room and a Douglas bag successively.

The air was analysed by the Haldane apparatus. The COHb content

of the blood was measured by the Hartridge reversion spectroscope(9). The blood samples were obtained from the tip of the tail, a mesenteric arterial arcade, or the right femoral artery. These samples had very nearly the same composition as the blood in the splenic artery, provided the circulation was good. When the circulation was bad the sample was taken from the heart.

The CO was prepared by treating pure sodium formate with pure sulphuric acid. The gas was led through a saturated solution of caustic soda and passed up a soda lime tower and collected over water in a glass container. Samples of the gas were frequently analysed and the only impurities found were nitrogen and oxygen, which occurred through some air being left in the apparatus.

Action of CO on the intact spleen. In all cases the splenic volume diminishes when the animal is allowed to breathe CO. The effect on the splenic beat is inconstant, but we have never observed after CO poisoning, however gradual, any increase in the amplitude of the splenic beat such as follows CO₂ asphyxia. As a rule the beats disappear. The effect on the blood pressure varies according to the rate at which the CO is absorbed by the animal. If given quickly the B.P. falls (Exp. 1). If given slowly the B.P. is little affected (Exp. 2); there is usually a slight rise followed by a slightly greater fall.

Exp. 1 (Fig. 1). Air containing 3 p.c. CO was administered to a cat under paraldehyde anaesthesia for 3' 50". During this time the spleen volume decreased from 4.8 c.c. to 2.5 c.c. and the B.P. fell from 140 mm. Hg to 33 mm. Hg. The splenic beats were abolished although the organ was not dead as was shown by its recovery to a normal state three hours later. The amount of COHb in the blood in the tip of the tail was 56 p.c. at the end of the CO



Fig. 1. See text.

administration and this was probably not much less than the maximum amount present in the blood, since the rate of elimination is slow and not more than 3 p.c. or 4 p.c. COHb

would have been eliminated by the time that the sample was taken. Essentially similar results were found under luminal.

Exp. 2. Air containing 0.92 p.c. CO was administered for 26' 7" to a cat under luminal anaesthesia when the concentration of COHb in the blood was 41 p.c. The spleen volume decreased from 5.73 c.c. to 3.74 c.c. The course of recovery under oxygen was observed. The effect on the n.p. was slight.

		Time from commencement		B.P.	Spleen vol.	Pulse
CO on	...	00'	00"	130 mm. Hg	3.73 c.c.	108
		8	7	134	2.62	106
		11	40	134	2.73	106
		12	43	118	2.65	105
		16	00	118	2.51	114
		25	23	124	1.42	115
CO off; O ₂ on		26	7	130	1.40	—
		26	40	136	—	—
		1 hour later		114	2.25	—

When recovery was complete the cat was fed with 25 c.c. of warm milk containing 0.3 gm. of luminal by stomach tube and left breathing air for 12 hours. Air containing 3.4 p.c. CO was then administered and the normal sudden fall in B.P. and a large diminution in splenic volume were observed.

Under artificial respiration, or other circumstances where the ventilation of the lungs is very different from cat to cat, the actual percentages of CO in the air breathed required to produce the opposite B.P. changes may differ considerably.

A third type occurred in about one-sixth of the experiments. The spleen contracted as in the other experiments, but the B.P. rose suddenly and remained about 40 mm. Hg above normal for two or three minutes when it fell rapidly below normal where it remained as long as CO was administered. This type of B.P. effect may be seen in Fig. 10.

The varying nature of the effect of CO poisoning on the B.P. when contrasted with the constant effect on the spleen at once suggests that the splenic contraction cannot be a passive (or even active) reaction to the change in blood pressure.

Controls on the connection between fall in B.P. and splenic contraction. To establish this connection we carried out experiments in which a fall in B.P. was brought about by stimulation of the peripheral end of the cut vagus and by hæmorrhage from the right femoral artery. The following variety of effects were obtained in different experiments. In each case anaesthesia was by luminal.

(a) The spleen contracted slowly from 4.3-4 c.c. after a sudden fall of 102 mm. Hg in B.P. due to stimulation of the peripheral end of the cut right vagus. Recovery took place.

(b) The spleen contracted from 5.2-3.6 c.c. coincidentally with a fall

in B.P. produced as in Exp. 3 from 140 mm. Hg to 30 mm. Hg. Recovery was rapid and parallel with that of the blood pressure.

(c) In one experiment there was the change shown in Fig. 2. This we

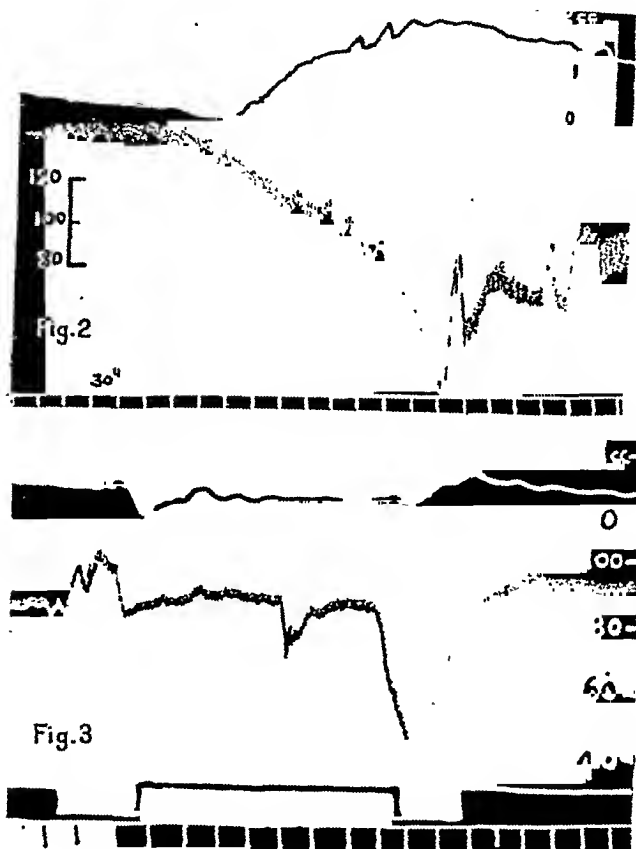


Fig. 2. See text.

Fig. 3. Decentralised spleen. At the 1st signal the median nerve was stimulated. At the 2nd signal the peripheral end of the vagus was stimulated. Time in 30 secs.

are satisfied was spontaneous, for there was no change in external conditions nor any hæmorrhage. The spleen dilated from 4.3-6.3 c.c. with an increase in the amplitude of the beats, coincidentally with a fall in B.P. from 140 mm. Hg to 20 mm. Hg. The recovery of the spleen began at the same time as that of the B.P. but it was not very closely parallel to it and it took slightly longer.

Experiments were also made on the spleen, decentralised in the manner described on p. 325. Fig. 3 is an example of the results. It will be seen

that a small rise of B.P. caused by stimulating the median nerve was accompanied by shrinking of the spleen and that a fall of blood pressure produced by vagal stimulation was accompanied by dilatation of the spleen. The latter effect is unusual, but it was obtained twice in spleens, the nervous connections of which with the central nervous system were undoubtedly severed, for stimulation of the splenic nerves central to the cuts had no effect. As will be mentioned later the fall of B.P. caused by acute CO poisoning does not produce any effect on the decentralised spleen (cp. Fig. 9).

From the results of Schafer and Moore(3) and also from our own experiments it is clear that a rise in B.P. may be contemporaneous with either a shrinking or with a dilatation of the spleen according to its mode of production.

The intravenous injection of Ringer's fluid results in a rise of B.P. and a splenic dilatation followed by increased amplitude of the beat (Fig. 4). Exactly the opposite effect holds when the animal is bled. In



Fig. 4. At the signal 20 c.c. of Ringer's fluid injected.

these cases, however, there is an alteration in blood volume which is responsible for the B.P. changes and which is probably responsible also for the changes in splenic volume.

Exp. 3. Spleen volume at commencement was 3 c.c.

Amount of blood withdrawn	Duration of withdrawal	B.P.	Splenic vol.
11 cc.	10"	100 mm. Hg.	2.6 c.c.
20	40	36	2.2
20	30	20	2.0

In other curves where the spleen was more active initially the abolition of the waves with hæmorrhage is often complete. As with CO poisoning, however, the only certain effect on the splenic beat is that they are never increased.

While a contraction of the spleen may be coincident with a fall in B.P. we cannot conclude from the simultaneous occurrence of a fall in B.P. with a splenic contraction that the two stand in the relation of cause and effect, since the foregoing experiments show that the spleen can contract or dilate simultaneously with either a rise or fall in B.P. under the same experiment in different animals.

We feel justified in assuming that the shrinkage of the spleen following CO poisoning is not due to the contemporary B.P. changes since the effect on the B.P. is variable and may be absent while the splenic contraction is a constant effect. Moreover in many of our curves the splenic contraction can be seen to begin a few seconds before the fall in B.P.

One other point requires discussion. Is the decrease in the volume of the spleen a contraction of the splenic muscle or is it due solely or in part to vaso-constriction?

(1) In Exp. 2 given above the pulse is almost unaltered and the B.P. is almost constant and on the whole shows a fall. This is inconsistent with a general splanchnic vaso-constriction, since if such were to occur and the pulse to remain constant a large rise in B.P. would occur. From this we conclude that there is no vaso-constriction in the spleen, for it is very improbable that a vaso-constriction in it should occur separately from a general one in the splanchnic area.

(2) An experiment on a dog under luminal in which the arterial arcades of the mesentery were prepared free from the intestine, but with a free circulation through them, and placed in a plethysmograph, failed to show any constriction of the vessels and possibly some dilatation.

We conclude therefore that the decrease in volume of the spleen which follows CO poisoning is due to a contraction of the splenic muscle and that the vessels of the spleen are not constricted.

The minimal amount of COHb in the arterial blood required to produce a splenic contraction. The mechanism of the splenic contraction is very sensitive. A contraction commences when the percentage of COHb in the arterial blood is only 8 p.c. and possibly this is not the inferior limit. We have compared the COHb concentrations in arterial blood and venous blood of the spleen and in spleen pulp. The method used for the spleen pulp was as follows:

The spleen was clamped off along the whole of the hilum as soon as a contraction commenced. It was then cut out of the body central to the clamp and specimens of splenic arterial and venous blood were drawn. The vessels to the spleen were cut off as close to the organ as possible

and it was then squeezed and massaged until it could be placed in a bowl of water without oozing blood for two minutes after being placed there. The spleen was minced and ground up lightly in 2 p.c. ammonium in a glass mortar. A sample of the fluid obtained by filtration from this preparation was analysed. This procedure was found to be necessary if all the arterial and venous blood was to be removed from the organ. With care considerable constancy in this treatment is possible. Experiments were made to ensure that this treatment did not remove COHb from a spleen, the pulp of which was known to contain COHb before the organ was excised. The last line of the table on this page shows the result of such an experiment.

Five experiments were made, luminal as usual being the anæsthetic. In Exp. 4 the animal breathed 0.1 p.c. CO. In Exp. 5 the animal breathed 4 p.c. CO and in Exp. 6 the spleen was not clamped off until one hour after the maximal contraction was obtained. Artificial respiration of air containing 0.2 p.c. CO was given. The results of the analyses were:

COHb content in terms of total Hb content of :

	Arterial blood	Spleen pulp Hb	Venous blood
Exp. 4	11 %	0.0 %	7.0 %
" 5	15	2.0	8.0
" 6	30	3.0	13.0
" 7	70	34.0	Sample unobtainable

The accuracy of the spectroscopic method is not great, but we estimate our probable error in these determinations at 1.2 p.c. COHb so that the differences in the above figures are significant.

It is quite clear that the spleen pulp does not contain COHb in significant amounts at the beginning or at the end of a contraction, and that the Hb of the splenic pulp does not contain CO until the spleen has been contracted for some time. From this lag in COHb content of the venous blood behind the arterial blood together with the long interval which elapses before COHb appears in the spleen pulp we conclude that it is possible that the contraction of the spleen results in the expulsion of unpoisoned red cells from the spleen sinuses or spleen pulp into the splenic veins and that in this way the extent of the CO poisoning of the blood in the general circulation is reduced. We have not, as yet, accurately mapped out the whole time course of the rates of CO poisoning of the arterial and venous bloods of the spleen and until this is done we would not wish to lay too much stress upon this view of the significance of the splenic contraction.

The purpose for which these experiments were made was to ascertain whether the spleen commenced to contract before it contained appreciable

amounts of COHb, and this is shown beyond doubt by these results. It is difficult therefore to believe that the splenic contraction is the result of a direct stimulation of the spleen either by CO or by the anoxæmia produced thereby.

The point of action of CO poisoning in producing a splenic contraction. The contraction of the spleen is, we have seen, an active response of the spleen to CO poisoning of the animal and may be the result of direct or indirect stimulation of the spleen or even due to an action of CO in causing the release of chemical substances, e.g. adrenalin, into the blood stream which cause the actual contraction.

Studies on the excised spleen. Our first experiments on this point were carried out on the surviving spleen. A cat or kitten was anæsthetised with A.C.E. mixture or with luminal and the animal bled to death from a cannula in the carotid artery. The abdomen was opened and the spleen rapidly removed by cutting through the tissues along the hilum. The spleen was promptly suspended in warm Ringer solution (10) in the apparatus described and a tracing of its movements taken. The apparatus was similar to that described by Dale and Laidlaw (11). The spleen was suspended with its long axis vertical by means of two glass hooks. By means of suitable taps we were able to bubble CO, N, air or oxygen through the Ringer solution in which the organ was suspended. The temperature was maintained at 36° C. by a gas thermostat.

The gas pressures were arranged so that no change in the rate or force of bubbling or in the size of the bubbles took place when the gas bubbled through the Ringer was changed. In this way all mechanical factors, which might otherwise have been responsible for the effects observed were removed.

The spleen was allowed to relax under a tension of 10 gms. until a constant length was obtained. When in this condition it was only very slightly contracted and the effect of passing nitrogen into the Ringer instead of oxygen was barely perceptible in either the rat's or in the cat's spleen. The effect of bubbling CO into the fluid was more marked and consisted invariably in a slight dilatation.

Similar results were obtained when the spleen was suspended in a 50 p.c. solution of the defibrinated blood of the same animal in Ringer, instead of in pure Ringer solution. In this case the CO was administered by changing the defibrinated blood for a similar solution of blood which contained 50 p.c. COHb. The solution of COHb was at the same temperature as the solution of oxyhæmoglobin and was introduced slowly into the bottom of the bath containing the spleen while the original solution

was removed at an equal rate from the top of the bath. In this way the fluids were changed successfully without any obvious disturbance of the spleen. The actual change of fluids was effected by means of taps adjusted so that fluid was removed from the top of the bath by siphoning at the same rate that fluid entered it under gravity at the bottom.

To make certain of these results the whole series of experiments was repeated. In this second series the spleen was first poisoned with adrenalin so that a contracted condition was induced which gradually passed off over a period of 50 minutes. In this case (Fig. 5) the spleen dilated rapidly when the oxygen or air was replaced by nitrogen or by CO. The same effect was obtained if the oxygen supply to the fluid in which the spleen was suspended was left unaltered and nitrogen or CO was bubbled into the fluid through a second jet. The more rapid dilatation which follows the administration of nitrogen we ascribe to the greater solubility of CO in Ringer and in the spleen than of nitrogen (Fig. 5). Since the excised spleen did not beat, the activity of the spleen was tested with adrenalin again after the experiment.

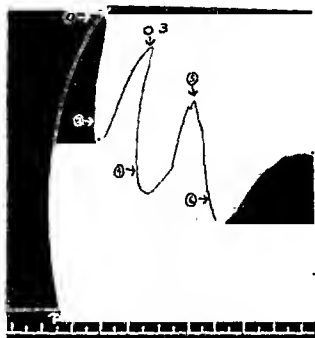


Fig. 5. Excised spleen of kitten. At the lowest arrow 4 c.c. of 1:50,000 adrenalin added. The numbers indicate the gas passed into the bath. (1) N_2 ; (2) air; (3) CO; (4) air; (5) CO + O_2 ; (6) air. Time in 2 mins.

The main conclusions which we wish to draw are: (1) that the surviving spleen does not contract on being poisoned with CO; (2) that the effects of CO on the surviving spleen do not differ in any essential detail from those of simple oxygen want as produced by the administration of nitrogen.

The action of CO in causing a contraction of the intact spleen must therefore be on some part of the body outside the spleen.

The possibility of a chemical mechanism. Excision of adrenal and pituitary glands. The adrenal glands were excised in cats under luminal anaesthesia. Fig. 6 shows that the excision had no effect on the result of CO poisoning. Air containing 4 p.c. CO was breathed. Breathing stopped and was replaced by manual artificial respiration of oxygen for 95 secs. The spleen subsequently recovered. This result has been

obtained many times and with more dilute mixtures of CO and air and a contrary result has never been observed. So that even if the adrenals



Fig. 6. Administration of CO (at signal) after excision of adrenals. 53 p.c. COHb in blood. Blood pressure fell from 85 to 35 mm. Hg. Spleen contracted 1.6 c.c. Time in 30 secs.

do secrete adrenalin into the blood stream under the influence of CO poisoning they do not play an essential rôle in the causation of the splenic contraction.

The pituitary body was removed by decerebration, the cut passing posterior to the pituitary body. Fig. 7 gives the effect. As a rule the B.P. falls as in the normal animal, but we have shown a tracing in which



Fig. 7. Decerebrate cat. CO administered at signal. At the arrow the arterial blood was 34 p.c. saturated with CO. Time in 30 secs.

the B.P. is constant in order to illustrate further the variability of the B.P. changes which can occur contemporaneously with a splenic contraction.

The normal reaction to CO poisoning is also obtained in a cat from which both the adrenal glands and the pituitary body have been removed.

The contractions of the spleen observed after adrenalectomy and hypophysectomy commence just as rapidly and in response to quite as small a COHb content of the blood as in the intact animal, and although in the case of the adrenals there are accessory glands which might conceivably produce the effect in the absence of the adrenals, we think that some quantitative differences should be observable in the two cases and this is not so.

That the contraction of the spleen seen in these experiments is due to a contraction of the splenic musculature is shown by the same arguments that were advanced on p. 318 for the same purpose in the normal animal. Certainly evidence is afforded in favour of the probability that these glands are not engaged in the causation of the splenic contraction following CO poisoning.

Effect of CO poisoning on the spleen after nicotine poisoning. An attempt was made to cut off the spleen from nervous connection with the body while leaving the circulation through it unaltered by poisoning the synapses with nicotine. The operation was made as described on p. 313. A cannula was placed in the right femoral vein and a 1 p.c. solution of nicotine was injected into this vein as required. In our first experiment the great rise of blood pressure produced by nicotine forced blood into the connecting rubber tube and caused rapid clotting. In the time taken to replace the cannula the paralysis by nicotine had ceased. To obviate this difficulty we used a glass cannula in the form of a T-piece. On the vertical limb of the T a bulb of suitable capacity (7 c.c. to 15 c.c.) was blown and this limb of the T-piece was connected to the manometer. In practice, when the rise in B.P. occurred, the blood flowed into the bulb which we had previously oiled with olive oil, displacing the sodium citrate with which the apparatus was filled into the manometer, and in this way no blood came into contact with the rubber tubing, so that no clotting occurred either then or when the blood returned to the artery as the B.P. fell to normal.

The animal was first poisoned with CO until a definite contraction of the spleen resulted. Recovery was then effected under oxygen. When recovery was complete nicotine was injected and the experiment was

repeated and in no case did a contraction of the spleen occur while the nicotine paralysis was complete. As a control, animals were subjected to two poisonings with CO separated by a time interval similar to that occurring in the nicotine experiments but without any nicotine poisoning so that the capability of the spleen to contract twice in response to successive poisonings with CO was clearly demonstrated. Fig. 8 illustrates this.

Exp. 8. A cat anesthetised with luminal was treated with air containing 10 p.c. CO until a contraction of the spleen occurred. Recovery was then effected under oxygen. When this was complete 25 mgm. of nicotine were injected into the right femoral vein at the first signal on Fig. 8 with the results shown. 1' 45" later a further 5 mgm. were injected. As will be seen, there was only a slight effect on the spleen and no effect on the R.R. Breathing ceased and artificial respiration was applied till the end of the experiment. 3' 45" later a further 5 mgm. were injected without effect. The paralysis was assumed to be complete and 3' 45" later air containing 10 p.c. CO was administered. This was continued for 3' 45" when the blood hæmoglobin was 46 p.c. saturated with CO. Although the recording apparatus was very sensitive no effect was observable. One half hour later the CO was again administered and a contraction of the spleen resulted immediately, indicating that the spleen had not been killed by the nicotine poisoning.



Fig. 8. See text. Time in 15 secs.

The enormous concentration of 10 p.c. of the CO and air mixtures administered requires explanation. The nicotine poisoning, if it is not to be fatal, cannot be made to isolate the spleen from the nervous system for more than 7 minutes. If a further dose of nicotine is given during this period with a view to prolonging it, disturbance of the tracing usually follows; moreover, the time of this second injection must be very accurately chosen. If it is given too soon death results. If it is given too late a contraction of the spleen from the action of the nicotine itself results. Moreover, if CO is still present in the blood in large quantities—e.g., over 20 p.c. COHb—when the nicotine poisoning begins to wear

off a contraction of the spleen from CO poisoning will result. Continuous injection of nicotine is impracticable.

Our object was therefore to obtain a sufficient concentration—40 p.c.—of COHb in the blood to ensure a contraction of the spleen taking place if one was obtainable under nicotine poisoning, and immediately this concentration was reached to remove so much of this CO from the blood by the artificial respiration of oxygen that the blood content of COHb was too low to have any marked effect on the spleen, all within the 7 minutes' duration of complete nicotine poisoning. Air containing 10 p.c. CO was found to be the most dilute mixture with which this was possible.

It must be realised that in no case were the animals allowed to breathe CO until their blood was in equilibrium with the mixture of CO and air administered. Indeed in very few cases was the blood of the animal anywhere near this equilibrium.

This result has been repeated many times without a different result ever being recorded. The fact that at the time of the second dose of CO (Fig. 8) the B.P. was only 50 mm. Hg is not of significance since contraction of the spleen has been observed to follow CO poisoning at much lower blood pressures than this. Nor, in view of our control experiments on the relation of the splenic contraction to the B.P. changes, do we regard the absence of effect on the B.P. as explanatory of the failure of the spleen to contract. That this result indicates that the contraction of the spleen is due to an effect of CO poisoning on the nervous system is made certain by the results obtained by perfusing the spleen and by decentralising it.

Decentralisation. (1) The spleen is exposed by an incision along the left nipple line from the last rib to the level of the anterior border of the ilium and is wrapped in cloths soaked in warm Ringer solution. These cloths are replaced frequently so that the spleen is always warm and wet. (2) The vessels to the spleen from the stomach are tied and cut. This is best done from the medial side. (3) The vessels to the pancreas are tied and cut, working from the tail to the head of the pancreas. (4) The splenic vessels and nerves are dissected from the lateral aspect from near the hilum of the spleen towards the mid-line until the veins are completely free and are traced to their junction as a single vein and the bifurcation of the splenic artery is reached. The dissection is then continued so that the nerves may be tied into a single bundle and cut. The splenic artery and vein are dissected and freed from the surrounding tissue to an extent which allows of their adventitiæ being removed over

a distance of quite 5 mm. This adventitia-free portion of the vessels is further freed from nerves by cauterisation with a crystal of silver nitrate. It is very important at this stage to ensure that the splenic artery and vein are quite free from the surrounding tissues both medial and lateral to their bifurcation. To ensure this it is frequently necessary to tie and cut the branches from the artery and to the vein connecting them to the duodenum, the head of the pancreas, and so on which have not been tied in the earlier parts of the operation owing to their inaccessibility before the main splenic vessels are dissected. Neglect of this precaution leads inevitably to disaster, when the next stage is performed. (5) A double ligature is passed under the splenic vessels and tied so as to include all the tissue remaining, other than the splenic vessels, which connects the spleen to the body. This tissue is cut between the ligatures; this procedure is difficult and requires considerable practice in order to obtain a healthy spleen. Complete decentralisation is effected by it.

By stimulating the nerves tied off it is possible to produce a contraction of the spleen after it is enclosed within the plethysmograph, and in this way the ability of the spleen to contract and whether it is still alive may be tested. A cannula is placed in the right external jugular vein so that adrenalin may be introduced to the spleen if necessary. Fig. 9 shows that there is no effect whatever on the spleen when the

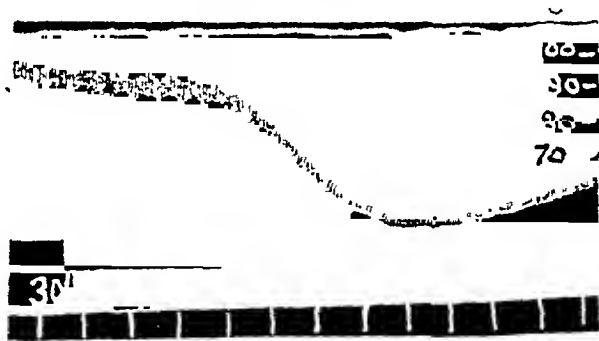


Fig. 9. Decentralised spleen. Administration of CO at signal. See text.

animal is poisoned with CO although the normal effect on the B.P. is present. The cat was anæsthetised with luminal and the spleen responded by marked contraction and ready recovery to stimulation of the splenic nerves isolated in the operation and to the intravenous injection of 1 c.c. of 1 : 20,000 adrenalin solution. Air containing 2.5 p.c. CO was

administered for 2' 16". The B.P. fell from 100 mm. Hg to 50 mm. Hg. The spleen failed to register any contraction at all, although its volume was 4.5 c.c. and the recording apparatus was very sensitive.

The effect of isolating the spleen from nervous influences of the rest of the body is definitely to prevent a splenic contraction following the poisoning of the animal with CO although the spleen receives poisoned blood in just the same way that an intact spleen would, although possibly in lessened amount. This cannot account for the result, because we have found that when the spleen is operated upon in exactly the same way as for decentralisation with the sole exception that the isolated nerves are neither tied nor cut, a splenic contraction follows CO poisoning as surely as in the animal with an intact spleen.

If this conclusion is true then it should be possible to obtain a contraction of the spleen on administering CO to the animal while the organ is left in nervous connection with the body but receives its blood from an external and unpoisoned source so that no COHb can enter it. This we have succeeded in doing in the following way:

Perfusion of the spleen. The apparatus and the method of operation will be described fully in a subsequent paper in this *Journal*. When the perfusion was fully established the nerves to the spleen were stimulated near the hilum of the spleen and if a normal contraction of the organ resulted it was assumed that the spleen was in a sufficiently normal condition for the further conduction of the experiment to be of value. In this way an organ was obtained through which a steady circulation of oxygenated Ringer was maintained and which was in normal nervous connection with the rest of the animal. Such a spleen will survive for 2 or 3 hours in a condition in which reliable experiments may be made upon it. As Fig. 10 shows, such a spleen contracts when the animal is poisoned with CO.

The animal was allowed to breathe air containing CO until its blood hæmoglobin contained over 40 p.c. COHb when recovery was effected with oxygen. Simultaneous records of spleen volume, perfusion pressure in the splenic artery and carotid B.P. were made.

Exp. 9. Cat under luminal. Air containing 3 p.c. CO was administered at the signal for 3 minutes when oxygen was given. Respiration normal. The B.P. rose from 100 mm. Hg to 144 mm. Hg during 3 minutes, and then fell rapidly in the next minute to 60 mm. Hg, subsequently falling gradually to 38 mm. Hg.

The spleen contracted from 6.3–4.4 c.c. The contraction commencing slightly earlier than the rise in B.P. The perfusion pressure began to rise when the splenic contraction was near to its maximum, rising altogether from 70 mm. Hg to 113 mm. Hg, falling before the B.P. fell to 74 mm. Hg and remaining at about this level.

Recovery was obvious within 7 minutes of the commencement of oxygen respiration. It was completed later on under artificial respiration. (Fig. 10.)

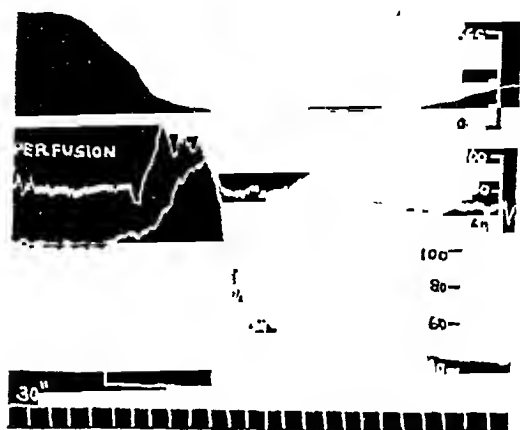


Fig. 10. Perfused spleen. See text. Perfusion pressure throughout is represented by the middle curve.

In most experiments of this kind the B.P. falls continuously from the commencement of CO administration. The spleen invariably contracts.

In some experiments the spleen was excised after the contraction, but before the CO administration was stopped, and minced in dilute ammonia. The solution of spleen pulp hæmoglobin obtained in this way was then examined spectroscopically for COHb. In no case was any COHb found.

Decapitation. From the foregoing results we concluded that the point of action of CO poisoning responsible for causing a splenic contraction lay in some part of the C.N.S. A rough attempt at localisation was made by observing the effect of sectioning the brain stem at various levels. So long, however, as the spinal cord is intact no change in the reaction of the spleen to CO poisoning is detectable.

Fig. 11 shows one of our results obtained from a cat under luminal in which decapitation was effected and the spinal cord cut at just below the third cervical vertebra. Air containing 4 p.c. CO was administered for 3' 10" when oxygen was given instead. It will be seen that the splenic contraction followed after a long latent period whereas the B.P. fall occurred fairly soon. At the point indicated by an arrow there was 31 p.c. COHb in the blood hæmoglobin.

The long latent period is a constant feature and is probably due to the very poor circulation present in the decapitate animal. The lag of

the splenic contraction behind the fall in B.P. is not a constant occurrence although it is often seen. However, the actual contraction of the spleen



Fig. 11. Spinal cat. Administration of CO at signal.

is quite normal in its nature and extent. In many cases the B.P. rises very slightly (about 10 mm. Hg), and then falls slightly (about 12 mm. Hg) and in some cases there is no effect at all. The B.P. never falls to so low a level as it does in the capitate animal even with the greatest concentrations of COHb in the blood compatible with life which have reached 65 p.c.

From these experiments we conclude that the centre or centres which respond to CO poisoning by effecting a contraction in the spleen are situated in the spinal cord below the level of the third cervical vertebra. Above (p. 318) we have shown that the diminution in the volume of the spleen in the experiments is due to a contraction of the splenic muscle.

The nature of CO poisoning.

That our effects are due solely to anoxaemia there is little doubt. We have been unable to detect a specific effect of CO on any excised organ. Haggard and others have shown that the effects of CO on various tissues are those of oxygen want only. The various results of this research have been imitated closely by the administration of mixtures of nitrogen and oxygen containing 5 p.c. or less oxygen instead of CO. The differences have not been significant. A contraction of the spleen follows the administration of nitrogen containing 5 p.c. oxygen and all three types of curve mentioned above, pp. 314 and 315 have been observed. The decentralised spleen fails to contract and the perfused spleen in normal nervous connection with the body does contract when this mixture of nitrogen and oxygen is breathed. Nicotine abolishes the

effect just as in CO poisoning. We conclude therefore that our results are due to the oxygen want produced by the displacement of the oxygen of HbO_2 by CO in the blood of the animal.

We think that the effect is due to this oxygen want acting by way of the spinal cord rather than to any possible change in the acidity of the general blood plasma. It is possible that in those cases where the splenic contraction is associated with a rise in B.P. that increased acidity of the blood plasma may be responsible, but the normal reaction to CO poisoning in which the spleen contracts and the B.P. either remains constant or else falls immediately and continuously cannot be the result of increased acidity in the general circulation. Of course the actual stimulus directly responsible for the contraction of the spleen may be one of locally altered acidity due to deficient oxidation within, or immediately surrounding, the cells which react to oxygen want by causing a contraction of the spleen, but this is a different thing from general hyperacidity of the whole circulation. Our reasons for this conclusion are:

(1) The splenic contraction commences when the concentration of COHb in the blood is as low as 8 p.c. It is hard to believe that this slight oxygen want could produce a general rise in the cH of the blood large enough to have produced the observed effects. Moreover, respiratory effects such as result from a general rise in cH are absent at this stage, and this is a powerful argument against such a rise.

(2) The most usual effect on the B.P. is either to cause an immediate fall or else to have no significant effect, whereas the inevitable effect of a rise in cH of the blood is a rise in B.P.

(3) As we pointed out in the beginning of this paper, there is probably no vaso-constriction and possibly a vaso-dilatation under the influence of CO poisoning. Whereas in CO_2 asphyxia there is always vaso-constriction.

(4) The splenic beats are never increased and are often decreased by CO poisoning. CO_2 asphyxia always causes an increase in the amplitude of the beats.

One other point arises. We have assumed that the effect is produced by way of the spinal cord. Our experiments as quoted do not remove the possibility of the splenic reaction being a reflex one. In this case the action of the CO would be on the sense organs and although we have not made the crucial experiment of cutting the posterior roots of the spinal nerves and administering CO to the animal we do not think this probable since the spinal cord is much more likely to be the seat of a mechanism so very sensitive to oxygen want than are any of the receptor

organs exposed to it. So far as we are aware there is no receptor organ as sensitive to oxygen want or to changes in cH as is the respiratory centre, and here we are dealing with a mechanism which responds to oxygen want at any rate as quickly as does that centre. Finally we think that a reflex due to a stimulus of this kind would be likely to affect the respiration before it would affect such organs as the spleen unless the receptors were in the spleen. This is not the case, since we obtained a contraction of the spleen in our perfusion experiments. Yet in our experiments the splenic reaction is at least as early in appearing as any other. Altogether the probability is that the CO anoxaemia stimulates a centre or centres in the cord. All these experiments have been repeated many times and in all nearly a hundred experiments have been performed.

SUMMARY.

1. When cats are poisoned with CO the spleen volume decreases. The B.P. falls if the rate of poisoning is rapid and either falls slightly or remains constant if the CO is slowly absorbed. In a few cases a sharp initial rise in B.P. takes place. The mechanism is very sensitive and responds to as low a percentage of COHb in the blood as 8 p.c.

2. The decrease in volume of the spleen is independent of the B.P. changes and is due to an active contraction of the splenic musculature.

3. CO poisoning does not cause any vaso-constriction in the spleen.

4. The excised surviving spleen does not contract, but dilates, when poisoned with CO. This effect is due to oxygen want.

5. The spleen does not contract in response to general CO poisoning of the animal when it is removed from nervous connection with the body either by nicotine poisoning or by decentralisation although in each case the spleen itself is poisoned with CO.

6. The contraction is not due to an effect on the adrenals or on the pituitary body.

7. The spleen perfused with oxygenated Riinger but in normal nervous communication with the body contracts when the animal is poisoned with CO.

8. The spleen of the decapitate cat contracts in response to general CO poisoning.

9. It is concluded that the spleen contracts owing to the effect of CO in producing an oxygen want in the spinal cord.

10. It is suggested that a function of the splenic contraction is to expel unpoisoned red cells into the blood and so reduce the proportion of COHb to HbO_2 in the general circulation.

We wish to express our thanks to Mr J. Barcroft for suggesting the problem and for much advice and the interest he has taken in the research. We also wish to thank Professor Langley for much advice and criticism and for the facilities he has placed at our disposal.

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LATENT GLANDULAR HERMAPHRODITISM.
NEW "UNBOLTING" EXPERIMENTS.
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LIPSCHÜTZ has shown with his co-workers Krause and Voss, that the hormonal effect of an ovarian graft in the male is favoured when the testicular mass is reduced experimentally. In experiments with intratesticular ovarian transplantation⁽¹⁾ all the cases in which one testicle had been removed, revealed a feminine hormonal effect and the time of latency was shortened. In other series of experiments with intrarenal ovarian transplantation⁽²⁾ into males which had been subjected to partial castration and in which the testicular mass consisted of a testicular fragment only, the experiments were also positive almost without any exception and the feminine hormonal effect was perceptible about two weeks after the ovarian transplantation, whereas there was *never* a feminine hormonal effect when both testicles remained untouched in the body of the engrafted animal. These results give support to the assumption of Steinach⁽³⁾ that there is an antagonism between the male and female sex glands or, as it would be safer to say, to the assumption that there is an antagonism between the graft and the sex glands *in situ*.

Steinach's supposition was that the antagonism of the sex glands is to be understood in a double sense. First, the development of the sex characters which are favoured by the hormones of one sex, is inhibited by those of the opposite sex; secondly, a "taking" and a survival of an engrafted sex gland is impossible when the gland of the opposite sex is present *in situ*. The second thesis of Steinach's assumption has proved to be unjustified. As early as 1910 W. Schultz, and afterwards Sand, Moore and Fisher, have shown that the gland of the opposite sex can survive for many months even when both glands are present *in situ*. Lipschütz and Voss have observed several cases of this kind in experiments with intrarenal ovarian transplantation (unpublished). Moore⁽⁴⁾ concluded from these results, that the assumption of Steinach as to an antagonism between the sex glands is "entirely superfluous and unwarranted" as far as concerns his material. But Lipschütz⁽⁵⁾ has recently described new facts which show beyond any doubt that the

criticism of Moore is not justified. Moore has not taken into consideration the possibility that the graft of the opposite-sexed gland, though taking and surviving even for several months, is inhibited in its *hormonic effect* by the glands *in situ*. This means that there would be in these cases a *latent* glandular hermaphroditism, and one would expect the hermaphroditism to become somatically manifest if, by some operative interference, the inhibiting action of the gland *in situ* is suppressed. This is what Lipschütz has done in his experiments. Two male guinea-pigs were engrafted with one ovary each from the same female. The first male which had a testicular fragment only revealed a feminine *hormonic effect* about twelve days after ovarian transplantation, whereas the second animal remained negative during seven weeks. Then, both testicles were removed from the second male and in less than ten days the feminine *hormonic effect* on the teats became manifest. It is like an obstacle or bolt being removed which so far had inhibited the manifestation of the feminine *hormonic effect*. This is why "*unbolting*" experiments seems to me to be the best designation.

It is evident that this experiment is of great importance for the problem of the antagonism. The unbolting experiment offers good opportunity for studying also in a more detailed manner the mechanism of the antagonistic influence between the sex glands. First of all there is the question whether in the unbolting experiment the time between the removal of the testicles and the beginning of the feminine *hormonic effect* is of the same duration as the time of latency in experiments in which the reduction of the testicular mass or castration is performed before the ovarian graft is made. The beginning of the feminine *hormonic effect* on the teats in unbolting experiments was observed⁽⁵⁾ even in less than two days after testicular castration in one animal and in three to six days in two other animals which were engrafted with ovaries as long as twelve weeks previously. But in these experiments (of which the details have not yet been published) the feminine *hormonic effect* lasted a very short time, only the beginning of mammary hypertrophy becoming visible. No definite conclusions could be drawn from them though they made it very probable that there is a difference between the time of latency in a previously castrated male and the time of latency after unbolting. Further, the question arises whether changes going on in the ovarian graft after the unbolting might be made responsible for the evoking the feminine *hormonic effect*. The first of these two questions was the object of the present experiments.

The experiments were performed in the following manner. Two young

males were engrafted with one ovary each from the same young female; transplantation was made into the kidney by a special new technic the details of which I shall describe elsewhere; the ovary was previously cut into several fragments. Both testicles remained *in situ*. Three to eight weeks afterwards both testicles were removed from one animal and the sequent condition of the teats was observed with the greatest care. The hypertrophy of the teats can be detected by an experienced observer in its very early beginning, especially by the characteristic hyperæmia of the teats and of the surrounding area which becomes also very soon protruded and shiny. These transformations are not induced by operative interference in the abdominal region as has been shown by hundreds of abdominal operations on the guinea-pig in this laboratory. Besides this, the testicular castration was made in all experiments by the serotal way. Further, the sequent development of the teats show whether there was a mistake or not in deducing a feminine hormonal effect from the early changes in the teats. The results of five similar experiments are given in the following table. In a the testicles were removed some time after ovarian grafting, in h they were left in the body.

TABLE I.

Exp.	♂ weight at operation gr.	Weight of ♀ furnishing ovary gr.	Number of ovaries engrafted	Castration — weeks after ovarian trans-plantation	Weight when castrated gr.	Feminine hormonal effect	Time of latency	Duration of observation weeks	Weight of removed testicles gr.
1 a.	190	195	1	3	290	+	1 week	5	0.35-0.3
1 b.	210	195	1	—	—	0	—	5	0.10-0.11
2 a.	280	125	1	3	310	+	3 weeks	7	0.98-0.9
2 b.	225	125	1	—	—	0	—	7	0.55-0.57
3 a.	185	160	1	8	415	+	4 days	>9	1.07-0.8
4 a. ¹	200	210	1	8	315	+	4 days	9	0.17-0.13
4 b.	195	210	1	—	—	0	—	9	0.63-0.69
5 a. ¹	175	205	1	7½	305	0	—	9	0.65-0.53
5 b.	170	205	1	—	—	0	—	9	0.81-0.76

¹ Twelve days after the first operation, the epididymis was resected in 4 a, and the vas deferens ligatured in 5 a. This was for other experimental purposes, and was of no, or of no durable, influence as to the feminine hormonal effect.

In Exp. 1 a the changes of the teats set in about a week after the removal of the testicles. The experiment was continued for two weeks after unbolting: the control animal (Exp. 1 h), with both testicles *in situ*, remained negative. In Exp. 2, with testicular castration three weeks after ovarian transplantation, the hypertrophy of the teats set in about three weeks afterwards. A week later both animals were sacrificed. The

control animal with both testicles *in situ* remained negative. In the third experiment there was no control animal, but nevertheless it is of the



Fig. 1.

Fig. 2.

Figs. 1 and 2. *Unbolting experiment (Exp. 2)*. Both guinea-pigs were engrafted with ovaries of the same female.

Fig. 1. Three weeks later No. 2 a was subjected to testicular castration. The photo shows the condition of the teats four weeks afterwards.

Fig. 2. No. 2 b, which had both testicles *in situ*, revealed no feminine hormonal effect. Normal male teats.

highest interest. Testicular castration was made eight weeks after ovarian transplantation and as early as four days afterwards the hypertrophy of the teats began. In numerous experiments with intrarenal ovarian transplantation into males with testicular fragments or completely castrated, a time of latency of such an extremely short duration was never observed, whereas as already stated, a still shorter time of latency was observed in some unbolting experiments. Since also in Exp. 1 the time of latency was a very short one and in Exp. 4 the time of latency was also, as in Exp. 3, only four days, it becomes highly probable that the conditions realized in the unbolting experiment are in favour of shortening the time of latency of the feminine hormonal effect. This can be explained in the following manner. Ovarian hormonal activity can begin only after the ovarian graft has really "taken" or, as we should suppose, after it has become vascularized. Now, in the unbolting experiment in which the antagonistic testicle is removed several weeks after ovarian transplantation, "taking" or vascularizing of the graft is already done. In an ordinary case of ovarian transplantation into a castrated or partially castrated male we have a complex time of latency consisting of two components: first the time needed until the graft has taken and, secondly, the time needed until the hormones which now begin to enter the circulation, have influenced the *substratum* in such a manner that

the hormonie effect is rendered manifest. On the other hand, in the unbolting experiment we have a reduced time of latency corresponding to the second component only; the graft has already taken and feminine sexual hormones probably circulate already in the body, and the time of latency will be in the unbolting experiment in general only the time necessary for rendering the hormonie effect manifest. This explanation implies indeed the assumption that hormone production is not inhibited in the ovary by the testicles *in situ*; such an assumption is justified since the histological appearance of an ovarian graft in a male without a feminine hormonie effect is often quite identical with that of an ovary of a male with a positive feminine hormonie effect, as already shown by Sand⁽⁶⁾ and as observed numerous times in our laboratory.

In Exps. 4 and 5 testicular castration was made seven and a half and eight weeks after ovarian transplantation. Exp. 4 was positive. The feminine hormonie effect set in, as in Exp. 3, four days after unbolting; the control animal remained negative. Exp. 5 was negative.

The experiments as related above, show clearly that the removal of the testicles favours the feminine hormonie effect. In both cases in which testicular castration was made eight weeks after ovarian transplantation the time of latency was, as already emphasised, only four days. It is highly improbable that during such a short time far-reaching changes in the ovary can be induced¹ and we must rather suppose that the inhibiting influence of the testicle concerned not the ovary, nor hormone production, but the substratum on which the hormones act. This is, however, as yet only a supposition. Some other facts which also are in favour of this supposition will be communicated later on².

The condition of the testicles in the cases mentioned is also of great interest. The testicular weight was in general normal; but there were some exceptions. In the control animal of the first experiment the weight of the testicle was very small, being only about half of what is the rule at a similar age. Microscopically the seminal tubules were found to contain spermatocytes but no spermatozoa were present. Since an intrarenal ovarian graft was present and since it was histologically identical with the ovarian graft of the positive animal (1 a) of this experiment, it becomes clear that even a testicle not yet in full spermatogenesis can

¹ It was lately proved in a definite manner by new observations in this laboratory that no changes are induced in the ovary after unbolting.

² Since then it has been shown in our laboratory that a testicular fragment also, even when containing spermatozoa, can be inhibited in its masculine hormonie effect by the ovarian graft though this is not always so. A similar testicular fragment when alone present in the body, will always cause a masculine hormonie effect.

exhibit its antagonistic influence against the feminine hormonal effect. The small testicular weight in Exp. 4 a is to be explained by the fact that in this case, for other experimental purposes, a resection of the cauda epididymis was made on both sides twelve days after ovarian transplantation. Lipschütz and Voss have shown that resection of the epididymis can produce as regards the feminine hormonal effect in experimental hermaphroditism an effect like that produced by partial or complete castration, but that the time of latency may be sometimes a long one. But in Exp. 4 a no feminine hormonal effect was found during six and a half weeks after resection and the fact that a feminine hormonal effect set in almost immediately after the small testicles were removed, shows clearly that the latter though reduced in weight were able to exert a certain antagonistic influence. The seminal tubules were shown microscopically to be infantile. This confirms the above statement that the inhibiting action of the testicle against the feminine hormonal effect seems to be not dependent upon completion of spermatogenesis¹.

Exp. 5 a may also be especially mentioned. A ligature of the vas deferens was made on both sides twelve days after ovarian transplantation and about one to three weeks later a transitory feminine hormonal effect was observed on the teats; but afterwards there was a regression to the normal masculine condition. At that time the testicles were quite normal; both could be felt in the scrotal sac. It was supposed that the normal condition of the testicles was alone responsible for the negative feminine result. But this was not the case in this experiment as was shown by the absence of a feminine effect when both testicles were removed about six weeks after the second operation.

The microscopical bearing of these experiments is not dealt with now as Mrs D. Švikul will give later on a full account of it.

SUMMARY.

Experimental proofs are given that those cases in which an ovarian graft "takes" in the male notwithstanding the presence of both testicles

¹ The question is quite a delicate one. As I have recently shown the antagonistic influence of the testicles can be nullified if they are fixed in the abdominal cavity without interfering with the normal blood supply of the testicle (unpublished experiments). At first it seemed clear that the troubled spermatogenesis as induced by the experimental cryptorchidism is the real cause of that, that the testicle loses its antagonistic capacity all the more as the testicles with resected epididymis in the former experiments of Lipschütz and Voss were retained also. But the Exp. 4 a shows that the position is evidently a more complicated one: a testicle with degenerated tubules is capable of producing an antagonistic influence. Light has been thrown on these at first sight so contradictory observations by new experiments with which we shall deal later on.

in situ, must be considered as *latent glandular hermaphrodites* in which the hermaphroditism becomes somatically manifest after the antagonistic influence of the testicle is suppressed. The simplest method of rendering manifest somatically the inter sexual condition of similar cases, is testicular castration (the *unbolting* experiment)

Four new similar positive experiments are communicated in which testicular castration was made three to eight weeks after ovarian transplantation. In those cases in which testicular castration was made as late as eight weeks after ovarian transplantation the feminine hormone effect became perceptible as early as four days after the *unbolting*. It follows from this that the time of latency of the feminine hormone effect after ovarian transplantation is a complex one. The components probably are (1) the time necessary for the taking and the vascularizing of the graft and its becoming able to produce hormones, and (2) the time necessary for influencing the somatic substratum until the effect is visible. The second component may be called the *reduced* time of latency.

Since an ovarian graft which in presence of both testicles *in situ* has no hormone effect, can be histologically identical with an ovarian graft that has a positive feminine effect, it is not very probable that the effect of unbolting is likely to change the condition of the ovary. Nor is the short time of latency in favour of such an assumption. It is probable that the hormone production in the ovarian graft may be normal notwithstanding the presence of both testicles, but that the hormone effect is inhibited by some antagonistic influence, on the part of the testicle, on the somatic substratum.

I am obliged to my pupil, Dr F. Lange, for valuable help he has given me in assisting at operations and dissections.

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ON SOME CONDITIONS AFFECTING THE PER-
FUSION OF ISOLATED MAMMALIAN ORGANS.
BY F. EICHHOLTZ (*Fellow of the Rockefeller Foundation*)
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IN a study of the mechanism of urinary secretion by the isolated mammalian kidney, Starling and Verney(1) suggested that the inability of the isolated organ to concentrate chloride was due to the absence of some substance normally supplied to the blood by the tissues. We attempted to test this hypothesis by perfusing the kidney *in situ* by means of the pump arrangement described by them (see Fig. 4 of their paper), our idea being to bleed an animal into the reservoir of the pump at the same rate as the pump was delivering blood into the animal *via* the renal artery. The blood would thus circulate through the whole animal, and possibly under these conditions, provided that the animal had been previously fed on a chloride rich diet, the perfused kidney might exhibit its lost chloride concentrating power. We encountered, however, at the outset of these experiments, the difficulty that no blood could be made to pass through the kidney. The following experiment is typical (Exp. 1).

Exp. 1. Dog. 9.5 kilos. Anaesthetised with morphia, CE and chloralose
0.95 gm. intravenously.

11.10-11.25	350 c.c. blood taken from animal, defibrinated and placed in reservoir of pump. 400 c.c. normal saline given intravenously
11.30-11.45	250 c.c. blood taken from animal, defibrinated and replaced. This was repeated
11.45	40 mgm. heparin intravenously. 10 mgm. heparin placed in pump reservoir
11.50	Right kidney exposed from back. Cannula inserted into renal artery. Temperature and pressure of supply of blood = 37° C. and 120 mm. Hg. No blood flow
12.40	Left kidney exposed. Cannula transferred to left renal artery. B.P. = 120 mm. Hg.; T. = 37° C. at cannula. No blood flow. Warm saline poured over kidney: no blood flow
1.20	Left kidney excised: artery pulsating: no blood flow

Clearly the pump was unable to perfuse defibrinated blood through the kidney *in situ* even when the pressure and temperature of supply were 120 mm. Hg. and 37° C. respectively. Furthermore, this inability persisted when the kidney was completely freed from its surroundings

by excision. In a second experiment, 3 gms of urea were added to the defibrinated blood in the pump reservoir, but again no flow of blood occurred. Corresponding experiments were performed using heparin as an anticoagulant instead of defibrinated blood, and precisely similar results were obtained.

This intense vaso-constrictor action of defibrinated blood has been encountered repeatedly by numerous investigators during attempts to perfuse the isolated kidney. The maximum blood flow given by Jacoby(2) is 33 c.c. per min. through a kidney weighing 28 gms., the pressure and temperature of the blood supplied being 135–140 mm. Hg and 36.5° C. respectively. Jacoby and v. Sobieranski(3) obtained very variable flows, for example, in one experiment 12 c.c. per min. through the kidney of a 13 kilo dog, the blood-pressure being 160–180 mm. Hg and temperature of blood 38° C., whilst in another experiment through the kidney of a 15 kilo dog they maintained a flow of 100 c.c. per min., the blood-pressure and temperature being 150–160 mm. Hg and 35° C. respectively. Unfortunately, the anæsthetic used during excision of the kidney is not stated, and it may well be, as was suggested by Pfaff and Tyrode(4) that this variability of blood flow was due to the use of different anæsthetics or different depths of anæsthesia during the preliminary operation. Indeed, Pavy, Brodie and Siau(5) found it essential to add chloral hydrate to the blood in order to overcome vaso constriction. Janeway, Richardson and Parke(6) showed that there were no vaso constrictor substances in circulating blood but that these developed rapidly when the blood was shed and were present in maximal quantities in serum and in defibrinated blood. Hirudinised blood and plasma, however, contained only minimal quantities. This fact readily explains the good blood flow obtained by Richards and Plant(7) in their experiments with rabbits' kidneys *in situ*.

In view of the relatively enormous blood flow through the kidney which Starling and Verney obtained when perfusing the isolated kidney with defibrinated blood by means of the heart-lung preparation, it seemed worth while to investigate further the reason for this marked difference.

Fig. 1 shows diagrammatically the arrangement of the apparatus used, the pump being connected as shown in Fig. 5 of the paper by Starling and Verney(1). By appropriate turning of the taps *G* and *H* the kidney *C* could be perfused by the heart lung *A* or by the pump *B*. Furthermore, the blood was made to circulate through both heart lung and pump whichever was actually perfusing the kidney at the time. This was

effected by means of the two tubes *I* and *K*, the former connecting the arterial side of the heart-lung with the pump reservoir, the latter connecting the arterial side of the pump with the heart-lung reservoir. Let

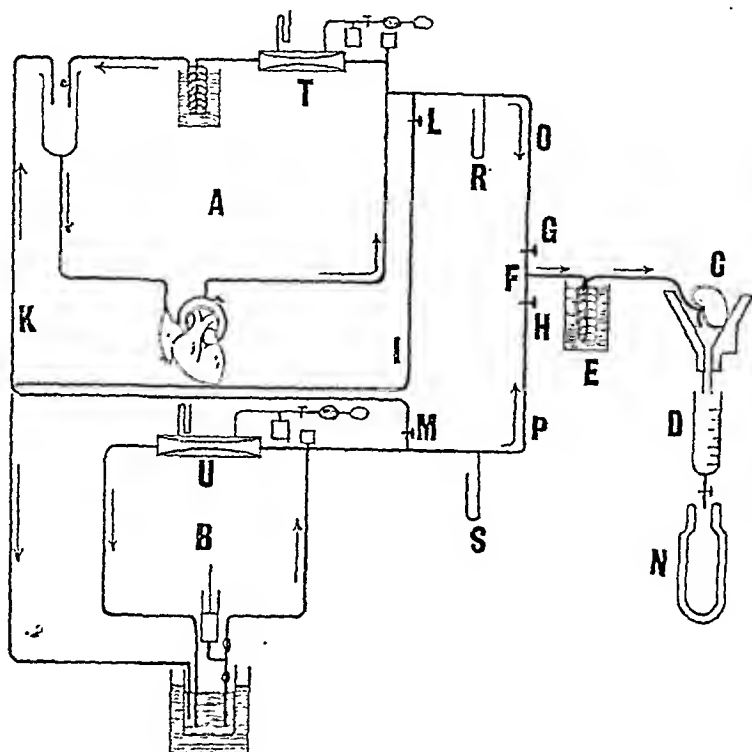


Fig. 1.

us suppose the kidney is being perfused by the heart-lung apparatus. Tap *G* is open and tap *H* closed. Blood thus passes along the tube *O*, through the warming coil *E* to the kidney *C*. It is collected in the Dewar flask *N* and poured back into the pump reservoir. The heart-lung is thus bleeding *via* the kidney into the pump reservoir. This loss of blood from the heart-lung is made good, however, by opening the screw clip *M* to such an extent as to allow blood to flow from the pump to the heart-lung at approximately the same rate as the rate of flow through the kidney. The course of the circulating blood may accordingly be represented as shown in the diagram *A*, Fig. 2.

By closing tap *G* and opening *H*, the kidney is switched directly to the pump, the return blood from the kidney in this case being poured back into the heart-lung reservoir. The loss of blood from the pump is made good by opening the screw clip *L* to an appropriate extent. The

circulating blood under these conditions follows the course shown in diagram *B*, Fig. 2. The blood-pressures in the two circuits were registered

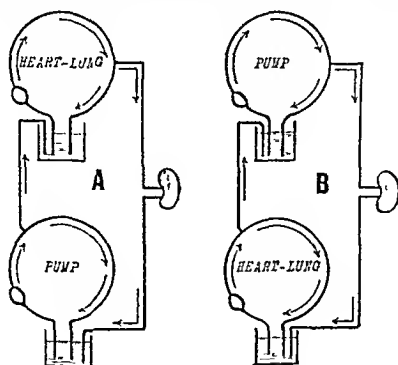


Fig. 2.

by manometers at *R* and *S* and maintained at the same level by suitable adjustment of the peripheral resistances *T* and *U*. That there is a marked difference in the rate of blood flow through the kidney according to whether the blood follows the course *A* or *B* of Fig. 2 is evident from Exp. 2.

Exp. 2.

Time	T° C.	B.P. mm. Hg.	Blood flow c.c./min.
2.22	37.0	120	79
2.55	"	"	91
3.10	"	"	103
3.24	30.5	"	136
Switch to <i>B</i> circuit (Fig. 2), the pump having just been filled from heart-lung circuit			
3.27	36.5	120	43
3.31	37.0	"	24
3.37	Switch to <i>A</i> circuit		
3.40	37.0	120	81
3.42	Switch to <i>B</i> circuit		
3.43½	37.0	120	86
3.44	"	"	48
3.50	"	"	22
3.53	Switch to <i>A</i> circuit		
3.57	—	125	81
3.58	36.0	"	86
4.02	Switch to <i>B</i> circuit		
4.04	36.5	130	63
4.06	"	134	53
Run in more heart-lung blood to pump reservoir			
4.08	—	—	61
4.13	—	110	52
Switch to <i>A</i> circuit			
4.15	—	—	97
Wt. of kidney = 39 gms.			

v. Frankenberg. He confirmed in general Krogh's results on the effects of hydrostatic pressure and on the semi-rigidity of the air sacs, paying special attention to variation in their volume. He found that sacs cut out of the body increased in size (almost entirely in their long diameter) when placed in a mixture of blood and water, that they shrank when placed in strong salt solution or when dried and then swelled on removal to water. In the living larva subjected to an increase of hydrostatic pressure he found negative buoyancy to be associated with decrease in the volume of the sac which enlarged again as neutral buoyancy was recovered and similar but opposite changes were observed when larvæ were subjected to a decrease of pressure. He concluded that the sp. gr. of the larva was regulated, mainly at any rate, by swelling and shrinking of a colloid substance between the spiral fibres of the air sacs but from experiments, partly on pupæ, he thought there was an additional means of regulation in the secretion of gas, a conclusion which he did not reconcile with Krogh's experiments on this point.

In Krogh's and v. Frankenberg's experiments the sp. gr. of the larva was altered relatively to that of the water by using a change of hydrostatic pressure to forcibly dilate or compress its air sacs and the recovery of neutral buoyancy depended on their return to the normal volume. In the following pages I show that if the sp. gr. of the water is altered relatively to that of the larva the latter responds by changing the volume of its sacs from the normal to an abnormal volume so as to restore neutral buoyancy. In concluding that the sacs are not mere passive containers but organs capable of active changes of volume I am in agreement with v. Frankenberg whose results I may say I only became acquainted with after completing the draft of this paper. I have not, however, found any evidence of regulation by gas secretion.

Reaction to changes in the density of the water. If the larvæ of *Corethra* are living in an aquarium of fresh water it is easy to dissolve sufficient common salt in the water to alter its sp. gr. materially without affecting the health of the larvæ. Suppose we add 0.5 p.c. of salt so as to raise the sp. gr. of the water from 1.000 to 1.003: the larvæ which were perfectly balanced in the fresh water become positively buoyant in the salt and have to swim downwards vigorously, but in a few hours the effect will disappear and the little creatures be suspended in neutral buoyancy as before. Evidently they have increased the sp. gr. of their bodies from 1.000 to 1.003. The change is not, I think, due to any significant extent to the absorption of salt, the course of events is the same if sugar be used to increase the sp. gr. of the water, moreover, as will be shown later

(p 318) a positively buoyant larva can increase its sp gr when living in distilled water. It seemed probable, therefore, that the adjustment of the animals' sp gr was effected by an active change in the size of the air sacs and to determine whether this occurred it was necessary to obtain a microscopic side view of the sacs in the living animal. Unfortunately its shape is such that when held gently in a compressor either the back or the belly, never the side, is presented to the microscope objective. A normal larva from fresh water was therefore introduced into the wide end of a drawn out glass tube together with some water and slid along till it just jammed in the narrowing channel, the tube was stuck with plasticine on to the stage of the microscope and a cover glass, supported by stage forceps, and carrying a hanging drop of immersion oil, was lowered on to it so as to minimise the optical distortion of the image. Careful rotation of the tube brought the sacs into the proper view plane and the outlines of two of them were then traced on paper with the help of a camera lucida. Next the larva was released from the tube and transferred to a vessel containing a salt water mixture of density 1.003. When the creature had adjusted its buoyancy to correspond to the new environment it was replaced in the glass tube and the outline of the same sacs traced afresh.

Fig 1 is a sketch of the outlines of the sacs of two larvae before being placed in salt solution (continuous line) and 3½ hours after being placed in salt solution (broken line) at which time neutral buoyancy had been attained.

Though the nature and sign of the change of volume are obvious it might be rash to have an estimate of its exact quantity on drawings made under such poor optical conditions, but, repeating the experiment a great number of times, I have always found a definite decrease in the volume of the sac after the animal has adjusted itself from fresh to salted water and an increase when an animal adjusted for salted water is restored to fresh water and allowed to adjust itself back to the original buoyancy.

The next step was to ascertain how the animals would behave in fluids of less sp gr than water. It was found that they would tolerate up to 6 p.c. of alcohol in the water without showing signs of sickness for several hours, when first introduced into such a light liquid they showed marked negative buoyancy but were able to reduce their sp gr well below 1.000 so as eventually to attain neutral buoyancy in it. Using the technique just described it was demonstrated that this adjustment involved an expansion of the air sacs which persisted as long as larva

remained in the low density liquid but disappeared after it was returned to an aquarium containing distilled water and allowed to resume its normal sp. gr.

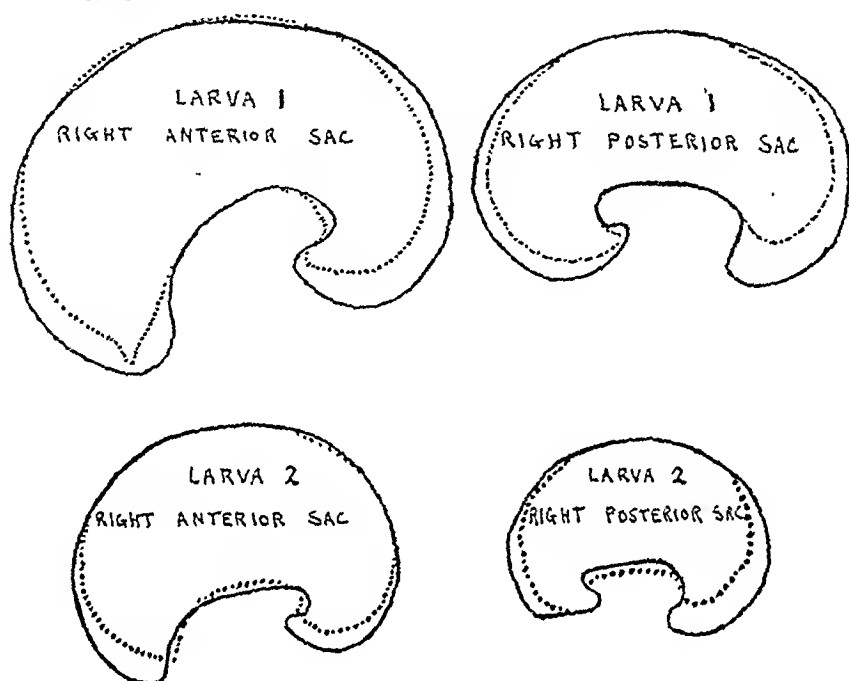


Fig. 1.

The full range of change of volume of the sacs can be demonstrated by keeping a larva in a light solution till it has made itself as light as possible and then sketching the sacs at full expansion; it is next transferred to a dense solution till it has made itself as heavy as possible when the sacs are sketched again, this time at full "contraction."

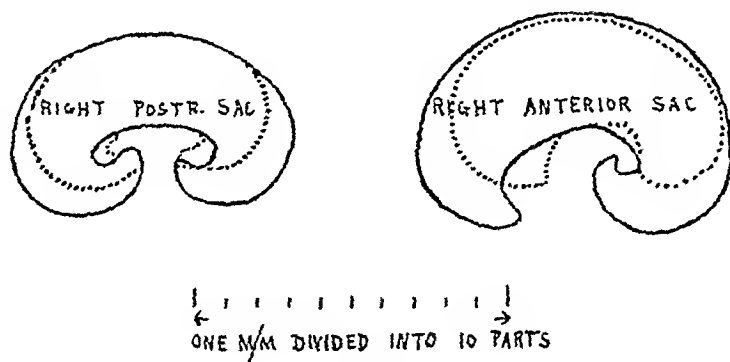


Fig. 2.

Fig. 2 shows the result of such an experiment. The larva was placed in a mixture of alcohol and water of sp. gr. 0.991 and left there till it attained neutral buoyancy when the sacs were sketched: it was then transferred to a salt solution of sp. gr. 1.003 where, after showing high positive buoyancy at first, it increased its sp. gr. and reached neutral buoyancy in $5\frac{1}{2}$ hours when comparison sketches were made. The continuous outlines represent the size of the sacs in the alcohol water and the dotted outlines the size of the same sacs in the salt water.

Volume changes of the same order of size are obtained if the larva is put into the dense solution first and the light one afterwards; in this case of course the sacs expand from the small to the larger size during the course of the experiment.

The amount by which the sacs can change their volume. One cannot measure the changes of volume of the sacs in the living animal with much accuracy under the microscope but the subject can be attacked indirectly. I find by experiment that the *Corethra* larva can adapt its buoyancy to liquids as dense as 1.004 or as light as 0.990 but these are approximately the limits. By making rough models in plasticine and weighing them an estimate was reached of the ratio between the volume of the sacs and that of the animal as a whole. Three determinations in different larvae gave 38, 44 and 57 respectively for the volumes of the air sacs when reduced to a common figure of 1000 for the volume of the whole body. Let us say that the mean ratio of the volumes is 46 : 1000. Now suppose a larva whose total volume is 1000 units to be in neutral buoyancy in water of density 1000. The volume of its air sacs (together) is 46 units and variable while the volume of its solid and liquid parts is 954 units and approximately constant. For this individual to increase its specific gravity to 1004 its total volume would have to be reduced by about 4 units, i.e. the volume of its air sacs must decrease from 46 to 42 units.

To suspend itself in liquid of density 0.990 it must decrease its sp. gr. to the same figure which would be done by expansion of the sacs from their normal 46 units to a new volume of 56 units (about). Hence it appears that each sac can shrink to about 91 p.c. or expand to about 122 p.c. of its normal volume. *Corethra* feeds on Entomostracans which are heavier than water and the effect of a meal must be definitely to increase its sp. gr. (Akehurst) hence expansion of the sac is called for after feeding, the processes of digestion being accompanied by a return to something like the normal volume.

Force required to alter volume of sacs. A bottle was filled with salt

solution of density 1.003 and an arrangement devised by which the pressure in it could be quickly raised or lowered. A larva was introduced into this dense fluid and of course started to float upwards. The pressure in the bottle was now rapidly increased (compressing the air sacs) till a point was reached where the larva was in neutral buoyancy showing that the sacs had been forcibly reduced to just that volume which made the larva's sp. gr. 1.003. This point was easily gauged, a slight further increase of pressure caused the larva to sink and a slight reduction to float upwards again. The creature's own efforts at compensation may be disregarded, there is no time for them to act, he would require an hour or two to bring its own sp. gr. up to 1.003 but the artificial compensation is done within one minute.

Similar experiments were done with fluids lighter than water when of course a negative pressure had to be applied. The results are set out below in the first and second columns, the third and fourth columns show the change in volume calculated (from the observed sp. gr.) to have occurred and the pressure which (by Boyle's Law) would suffice to produce such change if the sac had no rigidity and were a mere flaccid bladder. The difference between the pressures set out in the second and fourth columns therefore affords a measure of the intrinsic resistance of the sacs to change of volume.

Sp. gr. of fluid	Pressure which compensated cm. Hg.	Calculated change of volume %	Pressure required if sac had no rigidity cm. Hg.
1.004	+36	- 9	+ 7
1.002	+17	- 4	+ 3
1.000	0	0	0
0.997	-23	+ 7	- 5
0.994	-33	+13	- 9
0.991	-53	+20	-13

Time taken to adjust buoyancy in light and heavy fluids. In the middle of its range of adjustment *Corethra* can correct its buoyancy within a time to be reckoned in minutes but near the limits of that range the process becomes, rather suddenly, very much slower. Considering heavy fluids first, if placed in salt solution of sp. gr. about 1.004 the larva on an average requires 5 hours to adjust itself but in a solution of sp. gr. 1.002 about 20 minutes suffices. Return from the high sp. gr. to the normal is more rapid, an individual which has taken 5 hours to raise its sp. gr. to 1.004 when restored to fresh water will compensate in an hour and 10 minutes. The difference is not so marked with the 1.002 solution but is definite, the increase in sp. gr. requiring about 20 and the return to normal about 14 minutes.

Turning to light fluids we find that not only is the range within which adjustment is possible greater, but the speed is higher over that range. Larvæ will reduce their sp. gr. from 1.000 to 0.991 in about 2 hours or to 0.997 in 15 minutes, but in this case return to the normal sp. gr. does not take a shorter time than departure from it. Increase of size of the sac is more rapid than decrease.

Permeability of the air sacs to gases. As already mentioned Krogh has shown that gases readily diffuse in and out of the sacs. Boycott and I have obtained striking proof of the rapidity of the process and the effect which a change in the dissolved gases in the water may have upon the buoyancy of the larva.

(1) If normal larvæ are put into air-free water the gases in their sacs diffuse away and disappear into solution in the water. A few larvæ were placed in water which had been completely freed from dissolved gases by boiling under reduced pressure, care was taken that the contents of the bottle though protected from contact with the air were exposed to atmospheric pressure through an elastic arrangement of the bung. At first the larvæ were in neutral buoyancy, two minutes later negative buoyancy appeared and grew more pronounced till at the end of an hour they could only raise themselves from the bottom of the bottle momentarily by the utmost exertion of their swimming powers. Next morning their condition was the same and examination showed that the air sacs had completely collapsed and were crumpled up like paper bags. Kept in air-free water larvæ remain lying helpless on the bottom till they die about the third day.

(2) If larvæ whose air sacs contain a certain gas are put into water containing a different gas, diffusion occurs and any difference between the diffusibility or solubility of the two gases is manifested by a temporary change of buoyancy, the effect of diffusion pressure acting on the air sacs. Larvæ introduced into water which had been freed from dissolved air and then saturated with nitrogen showed a phase of negative buoyancy lasting 25 minutes but then acquired and maintained their usual neutral buoyancy and lived, apparently normally for several days.

The primary sinking of the larvæ may be referred to the greater diffusibility and solubility of O_2 than N_2 so that, for a time, the air sacs lose the one gas faster than they gain the other. The results with hydrogen were still more striking. Larvæ which had been living for 24 hours in water saturated with nitrogen, and whose sacs presumably contained that gas only, were transferred to hydrogen saturated water. They showed marked positive buoyancy within one minute of the change;

solution of density 1.003 and an arrangement devised by which the pressure in it could be quickly raised or lowered. A larva was introduced into this dense fluid and of course started to float upwards. The pressure in the bottle was now rapidly increased (compressing the air sacs) till a point was reached where the larva was in neutral buoyancy showing that the sacs had been forcibly reduced to just that volume which made the larva's sp. gr. 1.003. This point was easily gauged, a slight further increase of pressure caused the larva to sink and a slight reduction to float upwards again. The creature's own efforts at compensation may be disregarded, there is no time for them to act, he would require an hour or two to bring its own sp. gr. up to 1.003 but the artificial compensation is done within one minute.

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Time taken to adjust buoyancy in light and heavy fluids. In the middle of its range of adjustment *Corethra* can correct its buoyancy within a time to be reckoned in minutes but near the limits of that range the process becomes, rather suddenly, very much slower. Considering heavy fluids first, if placed in salt solution of sp. gr. about 1.004 the larva on an average requires 5 hours to adjust itself but in a solution of sp. gr. 1.002 about 20 minutes suffices. Return from the high sp. gr. to the normal is more rapid, an individual which has taken 5 hours to raise its sp. gr. to 1.004 when restored to fresh water will compensate in an hour and 10 minutes. The difference is not so marked with the 1.002 solution but is definite, the increase in sp. gr. requiring about 20 and the return to normal about 14 minutes.

point of neutral buoyancy (overshoot the mark) before finally reaching and maintaining it, no doubt this is due to the animal setting its own special mechanism in motion and superimposing its effect on those of the purely physical forces acting in the same direction

A number of experiments were done in the same way with different gases, they need not be described in detail but for the sake of convenience all the results obtained are set out in tabular form above

From the above it appears that the sacs are permeable to oxygen, nitrogen, hydrogen, nitrous oxide, and carbon dioxide, and that a *Corethra* larva transferred to water containing a gas which is either more soluble (*e g* nitrous oxide) or more diffusible (*e g* hydrogen) than that contained in its air sacs becomes temporarily inflated and forced towards the surface, the effect being reversible. Once the diffusion effect has ceased and the tensions and partial pressures of the gases in the sacs have become the same as those in the water the animal assumes full control of itself, and despite the unusual gaseous environment and the novel contents of its air sacs, balances perfectly and behaves just as though it were in ordinary pond water

The question naturally arose whether with such strange gases the larva retained its power of adjusting itself to changes in the density of the water and we found that it did. Three larvæ were put into each of five bottles containing water saturated with different gases and a little of the free gas and allowed to remain there overnight. Next day they were in normal equilibrium and (with proper precautions to avoid admission of air) sufficient salt was added to each bottle to bring the density of the water up to 1.002, the larvæ thus brought into positive buoyancy were watched and the time noted when two out of the three (2/3) or all of them (3/3) had restored themselves to neutral buoyancy. The results are set out below

Gas	Time required to compensate to fluid of density 1.002	
Nitrogen	2/3 in 36 minutes	3/3 in 98 minutes
Oxygen	2/3 in 21 "	3/3 in 103 "
Hydrogen	2/3 in 42 "	3/3 in 99 "
Nitrous oxide	1/3 in 60 "	Remainder not compensated in 150 minutes
Air (control)	2/3 in 46 "	3/3 in 51 minutes

The times taken by different larvæ to compensate for new conditions vary, however, so widely that no importance can be attached to the different rates recorded for the different gases above, except as to nitrous oxide where the retardation seems significant of that gas having some poisonous action

The power of compensation to small changes of pressure was also

tested and found to be retained in each of the above gases though, again, in nitrous oxide it was very much slower than in air or the other gases.

Reaction to changes of pressure. In my experiments, as in Krogh's, matters were arranged so that the tension of the dissolved gases in the water remained at one atmosphere absolute whatever the changes in hydrostatic pressure: it follows that the pressure of the free gas in the permeable air sacs cannot have been maintained at any other pressure than one atmosphere and the walls of the sac alone had to support or react against whatever hydrostatic pressure was applied.

In the case of *Corethra* larvæ from shallow ponds in the Isle of Wight, I find that no visible effect is produced on the buoyancy by pressure changes less than ± 6 cm. of Hg, hence in pursuit of its prey or for concealment the larva can swim two or three feet upwards or downwards without disturbing its delicate hydrostatic equilibrium. An increase of pressure of $+15$ cm. Hg immediately brings larvæ into marked negative buoyancy but the compensating mechanism, whatever it is, comes into action and restores neutral buoyancy in about 10 minutes. Increasing the pressure from atmospheric to $+30$ cm. Hg produces a similar effect except that it takes about an hour for the average larva to regain neutral buoyancy while an increase from atmospheric to $+45$ cm. Hg is generally beyond the creatures' powers of compensation; they remain permanently too heavy and in many individuals the air sacs collapse just as they do in air-free water; in other cases the distortion accompanying collapse ruptures the semi-rigid wall allowing the body fluids to flow into the sac which may then resume its original shape and appear almost normal though in reality waterlogged and useless.

It may be remembered that expansion of the sac is found to be more rapid than contraction when the larva adjusts its sp. gr. to correspond with changes in that of the water, the same rule holds good when it restores the volume of its sacs after they have been compressed or expanded by an artificial change of pressure. A larva was placed in a bottle and the pressure raised to $+15$ cm. Hg causing negative buoyancy. After a few minutes the larva had restored its neutral buoyancy. The pressure was then abruptly reduced to atmospheric. This brought the larva into positive buoyancy and again it had to adjust its weight, this time in the opposite direction and when the process was complete the pressure was again raised to $+15$ cm. Hg and so on. The time taken by the animal to compensate for each change of pressure was noted.

(There is no difficulty in gauging the end point with a healthy larva working well inside its possible range of compensation, but if the animal

is tried to the uttermost, for example by making it compensate to a pressure of + 40 cm. Hg the last stage of the process is so long dragged out that one cannot accurately judge when it is complete.) The results were as follows:

Time required to compensate from atmospheric pressure to +15 cm. Hg		Time required to compensate from +15 cm. Hg to atmospheric pressure	
(a)	12 minutes		29 minutes
(b)	13 "		23 "
(c)	19 "		23 "
(d)	16 "		22 "
(e)	14 "		28 "
Average			25 minutes

Mechanism of adjustment. The experiments recorded in this paper give instances in addition to those given by Krogh and v. Frankenberg that when the relative sp. gr. of the larva of *Corethra* and its medium is disturbed a change takes place in the larva by which its sp. gr. becomes that of the medium. It may fairly be concluded that within certain limits of change and in non-poisonous media this adjustment of the sp. gr. of the larva always takes place. It does not seem possible to account for it in the different conditions except on the theory that sinking or rising through the water sets up sensory impulses bringing a regulating mechanism into action. Such sensory impulses might obviously be set up by the sensory hairs described by various observers and recently by Akehurst(3). The nature of the mechanism is, however, still obscure. Krogh's theory that fluid is pumped in or out of the air sacs is untenable for neither Krogh, v. Frankenberg, Akehurst or myself find any fluid there; moreover, in adjustment to light fluids the sacs increase in size instead of decreasing as they would on Krogh's theory. My experiments and the previous ones of v. Frankenberg make it, I think, clear that adjustment is effected by a variation in the size of the air sacs. This variation is not caused by muscular action. Krogh's experiments give strong evidence against its being due to gas secretion. We are then driven to conclude that it is due to a physical change in the wall of the air sac. Whether this change is in the semi-chitinous coat as supposed by v. Frankenberg or in the outer cellular coat is, I think, an open question and nothing can be said with any certainty as to the method by which sensory impulses give rise to the physical changes in the wall of the air sac.

The experiments on the effects of such gases as hydrogen, nitrogen, etc. were done in conjunction with Prof. A. E. Boycott, to whom I am

indebted for helpful criticism and advice besides allowing me to work in his laboratory. I am also indebted to the editor for criticism and help.

SUMMARY.

(1) As is known the aquatic larva of *Corethra plumicornis* possesses four closed air sacs or internal buoyancy chambers and uses them to maintain itself in exact hydrostatic equilibrium with the water in which it lives.

(2) Artificial variations of the density of the water disturb this equilibrium, but only temporarily, for between the limits of 1.004, and 0.990 the larva can, and does, adjust its sp. gr. to correspond exactly with the density of its environment. It increases its sp. gr. by reducing the size of its air sacs and decreases it by dilating them.

(3) It retains the power of doing this even when the only gas present in the environment or in the air sacs is hydrogen, nitrogen or oxygen.

(4) Reasons are given for concluding that the sacs themselves and not their contents are the active agents in such changes of volume.

(5) Compensation to changes of pressure as originally described by Krogh is discussed and it is shown that the hypothesis put forward to account for his facts becomes unnecessary if the above conclusions are correct and that it could not account for the new facts concerning adjustment of the larva to changes of density in its environment.

(6) The permeability of the sac and body walls of the larva to gases dissolved in water is illustrated by experiments showing the violent diffusion effects which ensue when an individual is transferred from water saturated with one gas to water saturated with another of different solubility or diffusibility.

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ON THE MIGRATION OF OVA IN THE RABBIT.

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As a general rule it is to be supposed that following ovulation, an ovum enters the Fallopian tube corresponding to the ovary of origin, and in bicornuate animals becomes implanted in that cornu of the uterus. In certain cases, however, it is found that ova become transferred from one side of the female genital organs to the other. The evidence of this appears to be largely observational and to rest on discrepancies between the number of corpora lutea in the ovaries and the number of foetuses in the corresponding horns. These discrepancies fall into two classes first, cases where corpora lutea are found in only one ovary whereas embryos are found in both cornua of the uterus, and secondly, cases where the number of embryos in one cornu is greater than the number of corpora lutea found in the corresponding ovary¹. The occurrence of these abnormal distributions indicates that one or more ova may on occasion migrate. This displacement could only come about in one of two ways. Either some ova on leaving the ovary wander across the body cavity and enter the opposite tube (external migration) or else some ova move down one tube, round the cervix and enter the other tube (internal migration). It is clear, however, that the actual specific anatomy of the uterus must limit the prevalence of migration. In the case of the rat and the rabbit each cornu has a cervical canal, that is to say, there are two cervical canals opening independently into the vagina, so that there is no direct communication round the cervix between the two cornua. In the rat and the guinea pig also the Fallopian tube has an ovarian capsule which would prevent external migration.

Migration in the rabbit, which is well authenticated (Marshall(3)),

¹ The question of the validity of the corpora lutea count as indicative of the number of ova discharged need not be discussed. Suffice it to say that the production of more than one ovum by one Graafian follicle and the production of more than one embryo by one ovum, the disturbing factors, are comparatively rare. On the other side of the question it should be remembered that the death of one or more embryos may prevent the detection of migration.

would seem, therefore, to be of the external type. In the pig, for which considerable data have been collected by Corner(1), migration might be of either type, but Corner has shown that while internal migration of the ova can be definitely proved in the pig, external migration has not yet been demonstrated. Corner goes so far as to state that one ovum in three migrates round the cervix, and that the peristaltic movements of the uterus have by some means the definite action of spacing embryos more or less evenly along the cornua. Migration, apparently, is almost always from the more crowded side to the less crowded. In view of this extensive work the existence and extent of internal migration in the pig is well authenticated, while external migration was not found, and if occurring at all, must presumably be rare in the pig.

A priori, external migration does not seem to be a very probable occurrence, depending as it would have to do on a combination of fortuitous happenings. Since an ovum has no means of auto-locomotion, the passage across the body cavity must be brought about solely by the movements of the intestines, and possibly to a lesser degree by currents in the fluid filming the peritoneum. It has been pointed out by Corner(1) that the peristaltic movements of the Fallopian tubes may perhaps set up suction resulting in the production of currents running into the infundibulum. Any such effect would, of course, be a very considerable help to a migrating ovum, but even when all possible assistance is taken into account it is clear that the means whereby an ovum may be carried across are far from reliable, and that the chances are all against an ovum which has missed the fimbriæ on the side of ovulation successfully completing the passage across to the other tube.

The evidence that external migration does take place is largely inferential. Certain abnormal formations of the human uterus make it possible to suppose that it occasionally happens in the human subject, and it appears to be the only type possible in the rabbit. Leopold(2) stated a long time ago that animals having an ovariectomy performed one side and a salphingectomy the other side would still continue to breed, presumably by means of external migration. The possibility, however, that external migration may often occur does not seem to have been strengthened by work such as that done by Corner, and for this reason it seemed desirable to undertake further experimental work on the subject, using some such animal as the rabbit where internal migration may be ruled out, to endeavour to obtain some idea of the frequency of external migration.

Experimental. The type of operation required to make breeding

conditional upon external migration is ovariectomy on one side and salpingectomy on the other. As the left ovary lies much lower than the right, and is, therefore, easier to get at to remove, the general operation was left ovariectomy and right salpingectomy. Such an operation is of necessity a severe one, and at the outset a similar kind of operation was performed on two animals to ascertain whether the disturbance caused by such operation in itself constituted any barrier to fertility. In these two cases an ovary was removed and the corresponding cornu severed just above the cervix. This operation, of course, would not prohibit conception in the intact horn provided that the consequent disturbance of the genital apparatus was not serious enough to cause sterility. By daily weighing, the detection of pregnancy was fairly easy, and within nine weeks of the operation both these rabbits were found to be pregnant. A laparotomy was then performed and both were found to contain perfectly normal foetuses in the intact horn. It is quite evident, therefore, that very severe operations can be performed on the reproductive organs of the female rabbit without causing disturbance sufficient to result in sterility.

Six does, varying in weight from 1.6-2.5 kgms, were then operated on as described above, unilateral ovariectomy and salpingectomy on opposite sides. Pregnancy could then only follow as a result of the ova travelling from the remaining ovary across the body cavity to the intact horn. When recovery was well advanced the does were mated with a buck which was known to be fertile, and by means of regular weighing a constant watch was kept for pregnancy.

Though the animals were mated continuously for 3-4 months and coitus was observed repeatedly, no pregnancy followed in any of the six rabbits, and when dissected at the end of this period no signs of pregnancy were found. At the same time, however, corpora lutea were found in the ovaries, showing that coitus and ovulation had taken place. In addition, it was observed that the intact organs presented a perfectly normal appearance. It must be supposed, therefore, that those does were sterile owing to non-migration of the ova, and hence, it may be concluded that what might be expected theoretically obtains in practice, that external migration of the ova, if occurring at all, is a phenomenon of considerable rarity.

Leopold's positive results may perhaps be explained on the grounds of regeneration of the excised ovary from a small remaining fragment, an occurrence which is known to be not uncommon. Nevertheless, observations on the distribution of corpora lutea and embryos in the rabbit (by

Hammond, for instance) suggest that migration across may occasionally take place.

SUMMARY.

Ovariectomy on one side and salpingectomy on the other side make breeding conditional upon external migration of ova. Six rabbits operated on in this manner were successfully mated for 3-4 months without becoming pregnant, and showed no signs of pregnancy when killed. Ovarian excision and salpingectomy on the same side was found not to prevent pregnancy. It is concluded, therefore, that external migration of ova is very rare.

The expenses of the work recorded above were defrayed from a grant from the Government Grants Committee of the Royal Society, to whom my best thanks are due.

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THE EFFECT OF CERTAIN CHANGES IN THE
PERFUSATE UPON THE ISOLATED AURICLES
OF THE RABBIT. BY E. COWLES ANDRUS¹.

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THE importance, to the regulation of the normal cardiac rhythm, of the presence in the perfusing fluid of certain ions in more or less definite concentrations, has been clearly established by the studies of many investigators. Among these the papers of Ringer⁽¹⁾, Locke⁽²⁾, Langendorff⁽¹⁾, Howell⁽⁸⁾, Mines⁽¹³⁾ and A. J. Clark⁽⁴⁾ may be cited. The mode of action of these ions has been variously explained. Most attractive is the idea of Mines⁽¹³⁾, who attempted to correlate the function of the ions of the perfusate with their action upon the aggregation of colloids *in vitro* and with their influence upon the charges on artificial membranes. Andrus and Carter⁽¹⁾ have described the effects of alterations in the hydrogen-ion concentration of the perfusing fluid upon the rate of development and transmission of the excitatory process. They have suggested that these effects may depend upon the part played by the hydrogen-ion in the equilibrium existing, across the cell membrane, between the cell contents and the tissue fluid. The present study was undertaken with a view to examining the effect upon the rhythm of the mammalian auricle, isolated by Clark's⁽⁵⁾ method, of certain changes in the ionic content of the fluid bathing it.

Method. The heart of the rabbit was removed and placed in cold Locke's solution. The pericardium was opened and the auricles were cut away at the A-V groove. The isolated auricles were then suspended in the apparatus described by Burn and Dale⁽³⁾ in Locke's solution at 37°. One appendix was fixed to a platinum pin on the lower end of a small glass tube extending down into the bath, and the other was attached by means of a thread to a writing lever. The Locke's solution was kept thoroughly oxygenated by passing a rapid stream of gas through the glass tube to which the preparation was attached.

The effect of changes in pH upon the rhythm of the auricle. The reaction of the solution was made to vary between pH 8 and pH 7 by means of

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two different acids. In the first series the Locke's solution was made up with .05 p.c. sodium bicarbonate, and the change was effected by adding various proportions of carbon dioxide to the oxygen bubbling through the bath, 3 p.c. CO_2 producing pH 7, pure oxygen pH 8. In the second series the solution contained .05 p.c. disodium hydrogen phosphate, in place of the bicarbonate, and the reaction was varied by adding normal phosphoric acid or normal sodium hydrate. The pH was measured with phenol red by comparison with a set of standards.

A distinct difference was observed in the effect of similar pH changes in the two series. Fig. 1 illustrates an experiment. The upper curve (A)

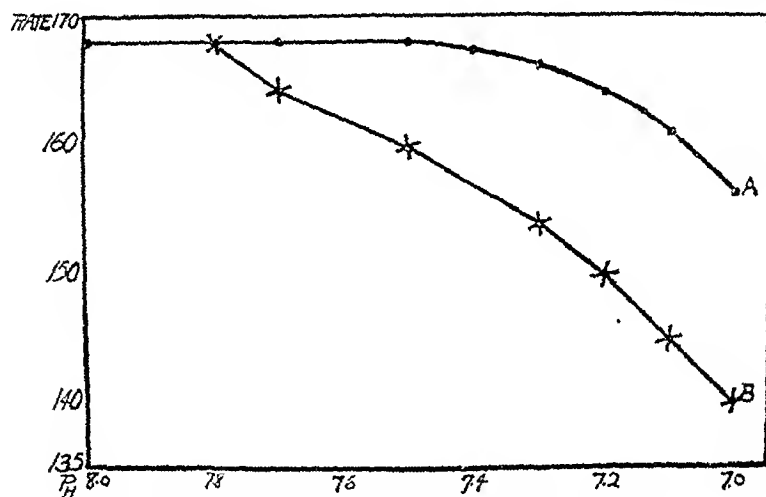


Fig. 1. Effect of changes in pH on auricular rhythm.

represents the changes in rate following changes in reaction from pH 8 to pH 7 when these alterations were produced with carbonic acid. The rate remained constant at 168 per min. until the pH had fallen to 7.5 and then gradually decreased to 156 at pH 7. Following similar changes in reaction, from pH 7.8 to pH 7, produced by adding phosphoric acid to the phosphate-buffered solution, the rate, as shown by curve B, fell steadily from 168 per min. at pH 7.8 to 140 at pH 7. The phosphate solution could not be made more alkaline than pH 7.8 without causing precipitation of the calcium.

These results appear to demonstrate a physiological difference between the two acids concerned, and they are of particular interest in view of recent work indicating peculiar properties of carbonic acid. Jacobs⁽⁹⁾, in reporting a study of the action of CO_2 upon infusoria and on the human taste buds, concludes that the greater effect produced by solutions of

carbonic acid, as compared with other acids at the same pH , is dependent upon the special ability of carbonic acid to penetrate the cell. In further experiments using the petals of *Symphytum peregrinum*, which contain a natural indicator, Jacobs(10) has confirmed his previous observations, and has shown that carbonic acid is able to enter and to raise the hydrogen-ion concentration within the cell even though it be introduced in an alkaline solution. This writer associates the physiological peculiarity of carbonic acid with two of its properties: "(a) the weakness of carbonic acid as an acid, permitting the existence of a relatively large amount of the free, undissociated acid in the equilibrium which exists at neutrality; and, (b) the readiness with which the undissociated acid or its anhydride, CO_2 , enters living cells." Scott(16) has shown that the respiratory centre is particularly sensitive to CO_2 , and Dale and Evans(6) have found, similarly, that the tone of the vasomotor centres is much more influenced by the carbonic acid content of the blood than by its cH . Hartree and Hill(7) have concluded that CO_2 is able to penetrate the cells of skeletal muscle and to raise their cH more quickly than other stronger acids.

Andrus and Carter(1) have suggested that the normal rhythmic development of excitation in cardiac tissue is dependent upon the cH of the fluid bathing it. The results reported above indicate something of the possible mechanism of this control. When phosphoric acid is added, we may suppose that the cH of the tissue fluid is raised without a corresponding rise in that of the cell content. With carbonic acid, on the other hand, the cH within the cell is presumably raised as well. The different effect produced by the two acids on the auricular beat could be explained upon the supposition that spontaneous rhythm is determined by the difference between the cH within and without the cell. Within the physiological range it has been shown that the contents of the heart muscle cell are more acid than the fluid bathing it (Clark(3)). An increase in the tissue fluid alone, by reducing the difference, leads to a slower development of the rhythmic excitation. When phosphoric acid is added to the solution surrounding the preparation, the cH of the bath tends to approach that of the cell, with consequent depression of the rate of excitation. With carbonic acid on the other hand, since the cH of the cell content rises as well, the effect of the same change in the external cH is less pronounced.

Effects of sudden changes in the concentration of electrolytes. Although numerous observations have been made dealing with the effects upon the cardiac rhythm of altering the concentration of one or more of the constituent salts of Locke's solution, the results of sudden, simultaneous

dilution or concentration of all these electrolytes have not, to my knowledge, been described. A series of observations was therefore made in which the concentrations of all the electrolytes in the fluid bathing the cardiac tissue were suddenly and simultaneously altered.

The isolated auricles of the rabbit were suspended in normal Locke's solution, thoroughly oxygenated, and having a reaction of pH 7.8. Changes in the electrolyte content of the bath were then made by the addition of measured amounts of certain solutions to the 100 c.c. of Locke's solution containing the preparation. Dilution was effected by adding distilled water or an isotonic solution of some non-electrolyte. Concentration was accomplished by adding a solution containing the salts of Locke's solution, except the bicarbonate, in the normal relative proportions, but in ten times the normal concentrations. These solutions were warmed to the temperature of the bath and were thoroughly oxygenated before being added. Neither dilution nor concentration caused any detectable change in pH. The changes in the electrolyte content are outlined in Table I.

TABLE I.

	+ 20 c.c. dist. H ₂ O	Normal	+ 2 c.c. 10 × Locke
NaCl	.71 %	.85 %	1.0 %
KCl	.035	.042	.050
CaCl ₂	.021	.026	.031
NaHCO ₃	.021	.025	.0245

The middle column gives the normal concentration of these salts. To the left are arranged the amounts present when the 100 c.c. of solution was diluted by 20 p.c. with distilled water or with a non-electrolyte solution and on the right the concentrations resulting when 2 c.c. of the ten-fold concentrated salt solution were added to the 100 c.c. of normal solution. The total concentrations of salts after these two procedures were 83 p.c. and 120 p.c. normal.

The sudden dilution of the Locke's solution in which the auricles were suspended had consistently a stimulating effect. The rate of beat was slightly but definitely increased and there was a sharp rise in the amplitude of contraction. This effect was, however, transient and usually passed off in from one to three minutes. That this result was not due to the lowering of the osmotic pressure of the bathing solution, incident to its dilution, was clearly shown by the fact that it followed as well when the diluent employed was isotonic dextrose (6 p.c.). A solution of urea, which since it penetrates the cells has no effect on the osmotic pressure, had also the same effect as distilled water. In Fig. 2 are shown portions of the record of an experiment. Section *A* is the record of the

beat in normal Locke's solution. Portion *B* is that obtained one minute after dilution by 20 p.c. with distilled water, and section *C* follows

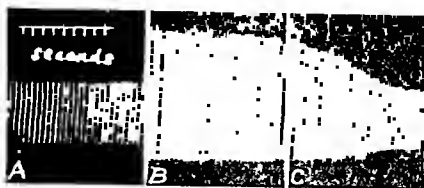


Fig. 2. Effect of dilution

two minutes later without further change in the solution. After the dilution of the Locke's solution the rate of beat rose from 116 to 128 per min. and the amplitude was conspicuously increased. Two minutes later, as shown in section *C*, both the amplitude and the rate had returned to the previous level.

Concentration of the electrolyte content of the bath caused, conversely, a distinct slowing of the rhythm and a reduction in the amplitude of contraction. This effect was also evanescent, passing off usually in three minutes. In Fig 3, section *A* shows the record of the beat in normal Locke's solution. *B* was taken one minute after the addition of 2 c.c. of 10 \times Locke, resulting in a fall in rate from 124

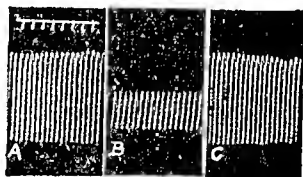


Fig. 3 Effect of concentration

to 110 beats per min., and a pronounced reduction in amplitude. In section *C*, taken three minutes after the change, the rate and amplitude have returned to practically the same as in the normal solution.

If the strength of the solution in the bath was changed several times in rapid succession, the rate and amplitude of the beat of the auricles changed in the contrary direction to the concentration of the electrolytes. In Fig. 4 are arranged portions of the record of such an experiment. The first section is that taken when the auricles were bathed in normal Locke's solution. Following dilution the volume of fluid in the bath was, in such cases, lowered to 100 c.c. by withdrawing a portion through the outflow at the bottom. Hence all calculations as to the amounts of

at pH 7.8. Stimulation of the vagus of the tortoise is likewise more effective in stopping the heart at pH 7 than at pH 7.8.

(5) Upon the isolated ileum of the rabbit, substances with a vagus-like action have a greater stimulating action at pH 8 than at pH 7.

(6) Upon the basis of these results it is suggested that the rate of development of the spontaneous excitation in the heart is dependent upon the difference in the hydrogen-ion concentration within and without the tissue, and that the susceptibility of the tissue to stimulation on the one hand or to inhibition on the other is dependent upon the same conditions.

I am indebted to the Medical Research Council for allowing me to work at the National Institute for Medical Research, Hampstead. I wish to thank Dr H. H. Dale for his kindly criticism and advice throughout the course of this investigation.

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STUDIES ON THE RESPIRATION AND CIRCULATION
OF THE CAT. IV. The heart output during respiratory
obstruction. By A. ST G. HUGGETT (*Beit Memorial
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In 1919 Barcroft, Boycott, Dunn and Peters⁽¹⁾ described a more direct method than previously used of measuring the cardiac output in the intact animal. A new method was thus available for investigating and controlling observations made in less direct ways. The following work was carried out to discover the effect of respiratory obstruction on the minute-volume and the stroke-volume of the heart, using the intact animal.

In 1914 Patterson and Starling⁽²⁾ investigated the heart output using the heart-lung preparation. They established a direct relation between the inflow per minute and the output per minute and the maximum output per minute and the pulse rate. They pointed out that their results were not directly applicable to the intact animal. Various methods of measuring the heart output in man have been devised by Krogh and Lindhard⁽³⁾, by Y. Henderson⁽⁴⁾ and by Douglas and Haldane⁽⁵⁾. Their results in regard to the output per beat are variable. None of them investigated the effect of respiratory obstruction on the cardiac output. The effect of respiratory obstruction on the respiratory movements and its relation to "Irritable Heart" was investigated by Haldane and his collaborators⁽⁶⁾ in 1919, but they did not enquire into the heart output.

In this paper an attempt will be described to discover the effect of inspiratory, expiratory, and double obstruction on the minute volume and the stroke volume of the heart. The results obtained show that the stroke volume varies enormously, that inspiratory obstruction increases both the minute-volume and the stroke-volume, while expiratory and also double inspiratory and expiratory obstruction combined cause the exact reverse.

The method. The principle of the method described by Barcroft and his collaborators is due to Fick⁽⁷⁾, and was afterwards used by Zuntz⁽⁸⁾. If O_2 be the total oxygen in c.c. used by the animal per minute,

blood was being taken. The total volume of air expired was measured with a spirometer.

The valves were now arranged as required to give obstruction to respiration, 30 minutes' breathing allowed and fresh samples of blood and air taken and analysed as before.

The results fall naturally into three classes, those obtained with inspiratory obstruction, those with expiratory obstruction and those with both inspiratory and expiratory obstruction simultaneously. In all the experiments the pulse rate was taken by counting on the artery or apex beat. For fast rates this method was found fallacious, therefore a series of experiments with varying respiratory resistances was performed in which the blood-pressure was recorded on a kymograph but no cardiac punctures were made. The average pulse rates were counted and divided into the average minute-volumes of the earlier experiments to give the average stroke-volumes. Some experiments were performed in which the pulse rate was recorded at the same time as the minute-volume was being taken in the same cat. The stroke-volume so obtained were in agreement with the previous experiments, confirming the assumption that the average of one set of experiments could be applied to the average of another set.

Inspiratory obstruction. Eleven experiments were made. A typical experiment is shown in Table I. It will be seen that in this case there was no difficulty in counting the pulse directly. There is an increase in both minute-volume and stroke-volume during obstruction, the former being due to an increase in oxygen consumption and a decrease in oxygen utilisation per c.c. of blood.

TABLE I. The heart output with inspiratory resistance.

	3.15	3.45	4.15	4.45	5.15	5.45
	p.m.	p.m.	p.m.	p.m.	p.m.	p.m.
Pressure in cm. H ₂ O	0	0	5	5	0	0
Temperature ° C.	37.5	37.5	37.5	37.8	37.8	37.6
Pulse rate per min.	160	165	145	152	170	163
Resp. rate	28	30	30	32	32	30
Resp. exch. per min. c.c.	887	1125	898	885	1378	1272
Expired air p.c. CO ₂	2.28	1.75	2.59	2.43	1.16	1.72
" " p.c. O ₂	18.22	18.85	17.92	18.11	19.12	19.17
P.c. CO ₂ excreted	2.24	1.71	2.55	2.39	1.57	1.68
P.c. O ₂ used	2.64	2.08	3.04	2.85	1.84	1.79
Apparent R.Q.	.81	.82	.84	.84	.85	.94
Corrected R.Q.	.77	.78	.81	.81	.82	.92
O ₂ consumed per min. in c.c.	23.4	23.4	27.3	25.3	25.4	22.8
O ₂ utilised in c.c. per c.c. blood = $\Delta - V$.0604	.0760	.0581	.0468	.0700	.0719
Minute-volume = $O_2/\Delta - V$ in c.c.	388	309	470	536	363	317
Stroke-volume in c.c.	2.43	1.88	3.24	3.52	2.14	1.94

In Table II is shown the average results of the ten other experiments, with the standard deviation σ , and the coefficient of deviation ν , which is the standard deviation as a percentage of the mean. Table II also gives the average pulse rate (obtained from kymograph experiments) and the stroke-volume (output per beat) with its percentage variation. The obstruction in all cases is the same, 5 cm. of water.

TABLE II Inspiratory obstruction 5 cm. of water. Average results of ten experiments

	Before	During	After
O ₂ consumption per min. in c.c.	21.81	23.9	21.6
O ₂ utilisation in c.c. per c.c. blood	0.682	0.533	0.722
Min. vol. in c.c.	332	457	310
" σ	91.5	106	56.5
" ν p.c.	27.6	23.3	18.2
" variation p.c.	—	+37.5	-3.6
Pulse rate per min.	162	156	172
Stroke volume in c.c.	2.04	2.93	1.80
" " variation p.c.	—	+43	-12

It will be seen that the minute-volume undergoes a 37.5 p.c. increase during inspiratory obstruction, while the stroke volume increases 43 p.c. the pulse rate remaining practically constant, varying only about 4 p.c.

Expiratory obstruction. These were in every way similar to those with inspiratory obstruction. The same type of valve was used with the difference that in order to expel the expired air the animal had to overcome a resistance of 5 cm. of water. Table III shows the results of a series of nine experiments. As before, the pulse rate was obtained with doubtful accuracy in some cases. It was overcome in the same way, a series of kymograph records being taken of the blood pressure and the average pulse rate being transferred to the average minute volume to give the average stroke-volume, giving the remaining results set out in Table III. The effect of expiratory obstruction is to diminish the minute volume and as the pulse remains nearly constant the stroke-volume is likewise diminished—an effect the exact reverse of inspiratory obstruction.

TABLE III Expiratory obstruction 5 cm. of water. Average results of nine experiments

	Before	During	After
O ₂ consumption per min. in c.c.	21.96	20.72	21.02
O ₂ utilisation in c.c. per c.c. blood	0.526	0.698	0.638
Min. vol. in c.c.	417	279	339
" σ	45.2	13.8	62.6
" ν p.c.	10.9	4.9	18.5
" variation p.c.	—	-33.1	-18.7
Pulse rate per min.	158	158	158
Stroke vol. in c.c.	2.64	1.76	2.25
" " variation p.c.	—	-33.1	-18.7

An interesting fact shown in Table III is that during expiratory obstruction rather less oxygen is taken up per minute than when at rest.

This is due to the fact that there is 33 p.c. less blood going through the lungs per minute, which is not wholly compensated for by an increase in percentage saturation of arterial blood.

Combined inspiratory and expiratory obstruction. The same technique was used as in the previous experiments. The obstruction to inspiration and to expiration was the same, viz. 5 cm. of water pressure, obtained by pushing the tube of the valve-bottles below the water for 5 cm. in both the inspiratory and expiratory bottles. Table IV shows the average values for eight experiments.

TABLE IV. Inspiratory and expiratory obstruction, each 5 cm. water.
Average results of eight experiments.

	Before	During	After
O ₂ consumption per min. in c.c....	22.06	19.56	23.86
O ₂ utilisation in c.c. per c.c. blood	.0506	.0625	.0564
Min.-vol. in c.c.	466	321	439
„ „	121.1	56.4	114
„ „ p.c.	26.0	17.5	26.0
„ „ variation p.c.	—	-31	-5.7
Pulse rate ¹	179	161	187
Stroke-vol. in c.c.	2.60	1.99	2.35
„ „ variation p.c.	—	-23.5	-9.5

¹ From kymograph experiments.

The chief facts shown by these results are that the effect of a double obstruction is to cause not a balance between inspiratory and expiratory obstruction but a fall in the minute-volume similar to the fall in output caused by the expiratory obstruction alone. Further, the variation in the minute-volume is not balanced by a proportionate variation in the pulse rate, consequently there is a 23 p.c. variation in the stroke-volume of the heart.

The above results on the stroke-volume were obtained on the assumption that the kymograph results recording the pulse rate were applicable in the mass to another series of cats in which the minute-volume had been measured. To check this assumption, six cats were examined in regard to the minute-volume and the pulse rate (measured with the kymograph) simultaneously, two for each type of obstruction. Three of these experiments are given in Table V.

DISCUSSION.

The experiments indicate that in urethanised cats, the heart is markedly affected by a respiratory obstruction of 5 cm. of water. It is worth noting that in breathing against this pressure the animals were performing a large amount of work and had a great difficulty in overcoming the obstruction. It was found that 7 cm. of water would cause

TABLE V.

	Before	During		After
		(1)	(2)	
<i>Exp. 1. Inspiratory obstruction.</i>				
O ₂ /min. in c.c. ...	19.55	18.10	21.60	15.14
(A - V) in c.c./c.c. blood	.0570	.0442	.0471	.0592
Min.-vol. ...	344	110	458	256
Pulse rate ...	200	162	192	200
Stroke-vol. ...	1.72	2.53	2.49	1.28
<i>Exp. 2. Expiratory obstruction.</i>				
O ₂ /min. in c.c. ...	19.25	13.41	14.12	18.90
A - V0601	.0691	.0672	.0610
Min.-vol. ...	321	194	210	310
Pulse ...	190	184	186	200
Stroke-vol. ...	1.69	1.05	1.13	1.55
<i>Exp. 3. Double obstruction.</i>				
O ₂ /min. in c.c. ...	21.62	19.88	18.81	20.70
A - V0636	.0688	.0679	.0649
Min.-vol. ...	340	292	284	320
Pulse ...	164	158	154	156
Stroke-vol. ...	2.07	1.85	1.84	2.05

asphyxia, the increased respiratory movement being insufficient to obtain oxygen or force out the expired air and it was gradually replaced by weakening respiration.

The cardiac output per minute is increased in inspiratory obstruction. This we might expect if we bear in mind the action of the respiration pump. Similarly, the output per minute is decreased in expiratory obstruction. But where we get both increased inspiratory and increased expiratory movements we do not have a balance between the two equal opposing obstructions, but a definite decrease in the output of 13 p.c. or more. In connection with this it is of interest to note that Douglas and Haldane(5) found that excessive dyspnoea produced by inspiring carbon dioxide caused a big fall in the minute-volume.

These variations in the output take place at the expense of both the oxygen consumption, and the oxygen utilisation. This latter is by no means a constant factor. The oxygen utilisation is held by Yandell Henderson(9) to be normally about 3-4 vols. p.c. at rest. In this paper it was only twice recorded as below 4 vols. p.c. The average resting value was 5.9 p.c. It falls during inspiratory obstruction, and rises during expiratory and double obstruction, the fluctuation being about 20 p.c.

The oxygen consumption increases during inspiratory obstruction as one might expect, seeing that greater inspiratory movements are carried out. But during expiratory obstruction and the double obstruction, the oxygen consumption falls, even though the respiration is deeper, probably more than in inspiratory obstruction. This is probably due to less blood going through the lungs. If the velocity falls appreciably it is obviously

the fact that the blood must take up less oxygen, even though the demand by the body be greater. This will be compensated for by a greater utilisation. The stroke-volume under these conditions increases by 43 p.c. during inspiratory obstruction, and decreases by 33 p.c. and 25 p.c. during expiratory and double obstruction.

The pulse rate shows very little variation under the conditions of the experiment, being approximately constant. Where tracings were taken of the blood-pressure it was found to be fairly constant, only changing 5-10 mm.

SUMMARY.

1. The minute-volume and stroke-volume were measured in urethanised cats during inspiratory, expiratory, and combined expiratory and inspiratory obstruction.

2. Inspiratory obstruction increases the minute- and stroke-volumes of the heart. It causes an increase in oxygen consumption and a fall in the utilisation.

3. Expiratory obstruction causes the exact reverse in all respects.

4. Combined inspiratory and expiratory obstruction causes a decrease in the oxygen consumption and in the minute- and stroke-volumes. The oxygen utilisation is increased.

5. The pulse rate undergoes only a slight change.

6. The circulation rate is approximately proportional to the output per beat.

In conclusion, I should like to record my thanks to Mr J. Barcroft for much advice, for facilities while at Cambridge, and for the loan of apparatus in London, enabling me to carry out this work. My thanks are also due to Professor Langley for permission to initiate this research in his laboratory; and to Professor J. Mellanby for criticism, advice and facilities.

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THE SIGNIFICANCE OF THE ACTION OF PITUITRIN ON THE SPLENIC VOLUME.

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SCHAFER AND MAGNUS⁽¹⁾ found that a diminution in the splenic volume followed the intravenous injection of extracts of the posterior lobe of the pituitary body. They did not say whether the diminution which they observed was due to the vaso constriction in the organ or to a contraction of the muscular capsule or to both. Dale⁽²⁾ has confirmed this observation and states that the muscular capsule of the spleen contracts after an intravenous injection of pituitrin although he gives no evidence for this outside the simple observation that the spleen volume diminishes. We have confirmed the diminution in splenic volume mentioned by these workers, but we have found that it is due solely to vaso constriction within the organ. We used a liquid extract prepared in the usual way from Messrs Armour's dry extract of the posterior lobe of the pituitary body. Our Ringer solution was prepared from the formula given by Dale and Burn⁽³⁾.

Our first experiments were made on the excised spleen. The apparatus used was similar to that described by Dale and Laidlaw⁽⁴⁾. The spleen was suspended with its long axis vertical in Ringer's fluid, maintained at 37.5° C by a thermostat. The fluid was freely oxygenated by a steady stream of air bubbles. The pituitary extract was added by means of a pipette to the Ringer solution in which the organ was suspended.

We were unable to detect any effect whatsoever following the addition of the pituitary extract. We experimented over a range of concentrations of the extract so that the splenic capsule was in contact with concentrations varying from 1/2,000,000 to 1/20,000 in different experiments, but in no case were we able to detect any effect, even after fifteen minutes. The sensitivity of both the spleen and the recording apparatus was tested by adding adrenaline. A concentration of 1 in 2,000,000 of adrenaline in the fluid in which the organ was suspended resulted in a contraction of the splenic musculature large enough to be recorded as a vertical line 13 cm. long on the tracing paper. The magnification by the recording

apparatus was $\times 37$ and the length of the spleen was 10 cm. Similar results followed the administration of pilocarpine. These controls were carried out upon every spleen upon which we experimented, after the pituitary extract had been given. From this we concluded that our apparatus was sensitive enough for our purpose. The pituitary extract was tested on the virgin uterus of the guinea-pig, suspended in the same apparatus. The response of this organ to a concentration of pituitrin of 1 in 1,000,000 was quite marked. The spleen muscle, therefore, is not contracted by pituitrin when it is administered to the excised organ.

We had still to show that the diminution in splenic volume obtained by Schafer and Magnus, Dale, and ourselves in the intact animal was not due to a contraction of the muscular capsule. The maximum diminution in splenic volume which can be produced by pituitrin is not so great as that which can be produced by adrenaline. For example, in an experiment where the spleen volume was 6.2 c.c. the maximal contraction which we could produce with pituitrin was 0.94 c.c. whereas with the aid of adrenaline we produced one of 2.74 c.c. The extent of this difference in the action of the two drugs varies considerably with the state of the organ, but it is always in the same direction. Since we know that adrenaline contracts the plain muscle of both the blood vessels and the capsule of the spleen it is conceivable that pituitrin acts only on one of these two, and since we know that pituitrin causes a constriction of the blood vessels it is probable that the difference in the extent of the effects produced by these drugs is due to an absence of action of pituitrin on the muscle of the capsule.

We were able to settle the question conclusively by perfusing the spleen. By using a pump which maintains a constant rate and output the mechanical conditions are so altered that the effect of vaso-constriction on the splenic volume is masked to a considerable extent. Whatever other effects it may or may not produce, pituitrin causes a vaso-constriction in the spleen, since its intravenous injection into the intact animal is followed by increased arterial pressure and decreased blood flow through the organ, the volume of which is diminished. While the blood flow does not diminish but temporarily increases when the capsule alone contracts after painting adrenaline on the spleen.

Wherever this vaso-constriction may occur it is clear that if the blood flow is maintained constant by increasing the arterial pressure the extent of the vaso-constriction will be diminished since the increased pressure in the constricted vessels will tend to dilate them. This is precisely what occurs in our perfusion apparatus and we have found that there are con-

centrations of pituitrin in the fluid circulating through the spleen which will cause a distinct diminution in the volume of the organ in the intact animal, while producing hardly any effect on the perfused organ, although a considerable rise in the perfusion pressure indicates that the drug is acting vigorously.

Moreover we cannot apply this argument to a contraction of the muscular capsule since such a contraction involves only temporary changes in blood flow and perfusion pressure. All that occurs is a temporary increase in blood-pressure at the actual time of the contraction, due to the venous congestion caused by the expulsion of blood from the organ by the contraction in addition to the ordinary blood flow. This increase does not end until the contraction is maximal, but the pressure returns to normal while the spleen capsule is still contracted. This is seen in the contraction of the spleen which occurs without simultaneous vaso-motor effects after CO poisoning(5). So that the total effect of perfusing a spleen with our apparatus will be to mask the effect of a drug producing vaso-constriction on the splenic volume. A diminution caused by contraction of the muscular capsule will not be masked at all. Consequently, to show that pituitrin does not cause a contraction of the muscular capsule we have only to show that the diminution in volume observed to follow its administration in the intact animal can be wholly masked in our perfusion apparatus and this we have done. We have experimented with various doses of pituitrin in this way and Fig. 1 gives an



Fig. 1.

illustration of our results. The spleen was in intact nervous communication with the body and failed to contract—a constant feature—on the injection of 1 c.c. of 1 in 1000 solution of pituitrin into the right external jugular vein. The effect of perfusing Ringer solution containing pituitrin in the concentration of 1 in 50,000 through the spleen is shown at the

second signal. The diminution in volume is very small, 0.2 c.c., and was 1.1 c.c. greater in a spleen of equal volume in the intact animal in which the concentration of pituitrin in the blood was approximately the same as in the perfusion fluid. At the third signal the organ was perfused with 1 in 1,000,000 adrenaline solution. This contraction involves both the splenic muscle and the arterioles and is 3.3 c.c. greater than the diminution in volume following the administration of pituitrin. A similar difference between the volume changes induced by the two drugs was observable in cases where the perfusion pressure rose to the same extent in both cases. About the time indicated by the first signal in Fig. 1 the cat died rapidly. The spleen contracted, and subsequently recovered. The period of recovery has been omitted from the figure. This effect is a constant one.

We conclude, therefore, that the volume decrease observed by Schafer and Magnus, Dale, and others was due to vaso-constriction, and not to a contraction of the muscle of the splenic capsule.

Description of perfusion apparatus.

The apparatus was designed for the purpose of perfusing the spleen *in situ*. The perfused fluid passes from the valves *V* to the bottle *A* where it is heated. From this bottle it may pass to the splenic artery either directly along the uppermost tube in the diagram (Fig. 2) or indirectly through the bottle *C*. The contents of this bottle may be changed without affecting the pressure at, or the flow through, the arterial cannula by suitable manipulation of the taps *E*, *F*, *T*, *G*. The drug which it is required to administer is placed in the bottle *C*, and is sent into the circulation simply by turning the taps *E*, *F*, *G*, so that the perfused fluid flows through *C* instead of through the tube connecting taps *E* and *G* direct. A screw clamp placed on the tube connecting taps *E* and *G* directly enables an adjustment to be made so that the linear rate of flow through, and pressure at, the arterial cannula is the same whichever route the circulation takes before reaching it. Bottles *A* and *C* are placed in heating baths and are fitted with thermostats (shown diagrammatically at *B*) which are so adjusted that the temperature of the fluid at the arterial cannula is constant. The returned circulation is to an artificial lung *H*, in which the fluid flows over small glass marbles which are aerated by air passing upwards through them from the tube *K*. From this "lung" the fluid passes back to the valves. In our experiments in this paper the perfused fluid was not allowed to return to the circulation. The spleen was perfused with Ringer, pumped from an aerated supply

contained in a reservoir *R* connected to the circulation by the tap *P*. Recording manometers are included in the circulation as shown. The

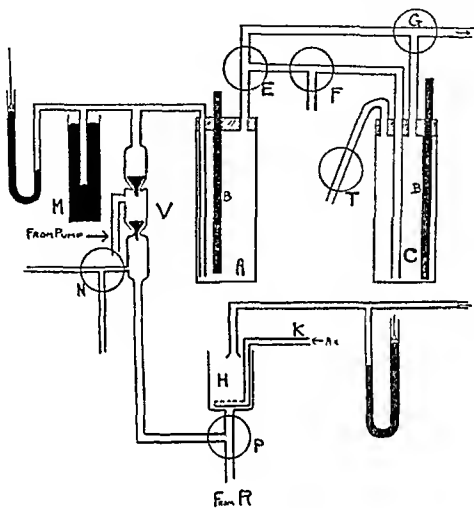


Fig. 2.

reason for placing the arterial manometer in the position shown is that if it were placed nearer to the artery some of the drugs perfused would diffuse into it, when on their way to the artery, and for a long time after bottle *C* is switched out of the circulation the drug would diffuse out of the manometer and into the organ. Owing to the position of the manometer our recorded pressures are always higher than the actual perfusion pressures. The difference is constant and is 11 mm. Hg in our apparatus. At *M* a mercury valve is interposed to prevent an unduly high perfusion pressure. The tap at *N* is for convenience in filling and washing the apparatus. The pump used worked at a constant rate and gave a constant output at all pressures, and the tubes of the apparatus were sufficiently rigid for there to be no significant variation in the flow through the spleen, when a vaso-constriction of any magnitude whatsoever was produced. The capacity of the tubes connecting the heating baths *A* and *C* to the arterial cannula is made as small as possible in order that the temperature of the fluid leaving these bottles does not have to be

blood became more venous, the alæ nasi showed marked respiratory contractions. The intravenous injection of adrenalin at once annulled the contraction of these accessory respiratory muscles. Similarly, we have observed that adrenalin apnoea is readily produced in the decerebrate cat. It is therefore evident that adrenalin apnoea is due to the local action of adrenalin on the bulb or mid brain and is independent of the advent of nervous impulses to the respiratory centre either from the cerebrum or periphery.

Cardio-inhibitory centre. In order to demonstrate the effect of adrenalin on depressor reflexes, the dose must be less than that required to produce a maximal adrenalin action. Otherwise the central depressor effects are obscured by the peripheral adrenalin action. The absence of effect of adrenalin on this centre is shown in the blood-pressure tracing (Fig. 1) obtained by a Gad manometer. This type of manometer was used to show cardiac effects rather than blood-pressure changes. 1 c.c. of .0033 p.c. adrenalin produced a rate of heart-beat of 212 per min. (A). The same dose of adrenalin immediately followed by central depressor stimulation resulted in a heart-beat of 128 per min. (B). Clearly then, adrenalin does not diminish the sensitivity of the cardio-inhibitory centre in depressor nerve stimulation.

Vaso-motor centre. The vagi were cut to limit the lowering of blood-pressure produced by stimulation of the central end of the depressor to

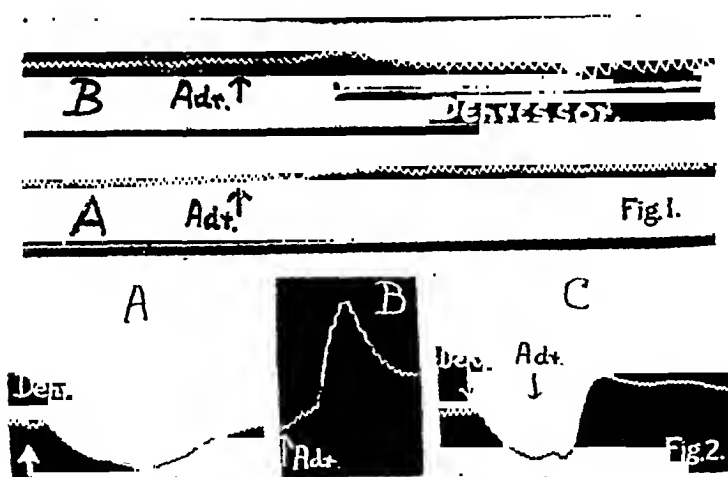


Fig. 1. Adrenalin on cardio-inhibitory centre. A, adrenalin alone. B, depressor stimulated after adrenalin.

Fig. 2. Adrenalin on vaso-motor centre (rabbit). A, depressor stimulated. B, adrenalin injected. C, depressor stimulated after adrenalin.

an effect on the vaso-motor system only. Fig. 2 *A* shows the fall of blood-pressure produced by central stimulation of the left depressor. Fig. 2 *B* shows the rise of blood-pressure produced by 1 c.c. of .001 p.c. adrenalin. Fig. 2 *C* shows first the fall of blood-pressure produced by depressor stimulation of the same strength as *A*, and, at the depth of the depressor fall, the rise of blood-pressure due to the injection of adrenalin of the same strength as produced the rise *B*. The rise produced by the adrenalin is approximately equal to the depressor fall. From the point of view which we are investigating, the adrenalin rise of blood-pressure may be a resultant of (*a*) stimulation of the peripheral vaso-motor nerve endings, and (*b*) the central inhibition of the vaso-motor centre in a manner comparable to that observed with the respiratory centre. But, since the adrenalin rise of blood-pressure during depressor nerve stimulation (*i.e.* during the period in which the vaso-motor centre is inhibited) is of the same height as when the depressor nerve is not stimulated it is evident that the adrenalin rise of blood-pressure is not the resultant of a central and peripheral effect but solely to the peripheral action of adrenalin on the nerve endings in the periphery. The experiments with the depressor nerve clearly demonstrate that adrenalin has no action on the cardio-inhibitory centre nor on the vaso-motor centre.

Pressor nerve stimulation shows precisely the same facts. The central end of the cut right femoral nerve of a cat was stimulated during the rise of pressure produced by the intravenous injection of .01 mgrm. of adrenalin (Fig. 3). The adrenalin rise of blood-pressure (*A*) was markedly

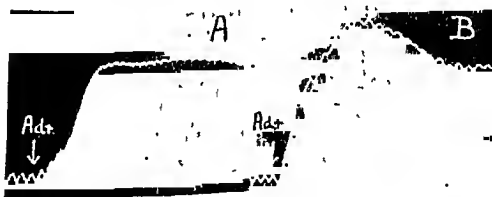


Fig. 3. Adrenalin on vaso-motor centre (cat). *A*, adrenalin injected. *B*, combined adrenalin injection and stimulation of femoral nerve.

increased by a simultaneous stimulation of the central end of the right femoral nerve (*B*), indicating that the bulbar pressor mechanism was unaffected by the adrenalin.

Swallowing centre. The experiments were made on a cat decerebrated

by Sherrington's method and on a cat anæsthetised by urethane. The stimulus to swallow was obtained either by placing a drop of alcohol on the fauces or back of the tongue, or by electrical stimulation of the central end of the superior laryngeal nerve. A graphic record was obtained by fixing a hook to the hyoid bone and attaching it to a recording lever. Fig. 4 shows the effect of an intravenous injection of

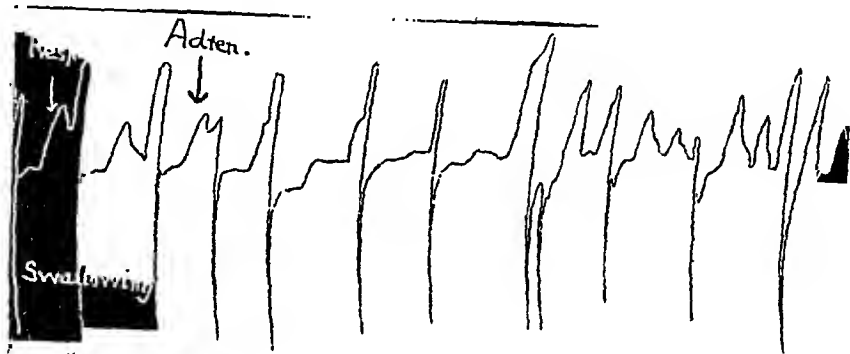


Fig. 4. Adrenalin on swallowing centre (cat). Large excursions=swallowing.
Small excursions=respiratory.

adrenalin on the swallowing reflex provoked by rhythmic stimulation of the central end of the superior laryngeal nerve. The small waves record respiratory movements, the large waves, swallowing movements. It may be observed that the adrenalin produced no effect on the swallowing reflex but almost abolished the respiratory efforts. After the apnoea passed off the tracing shows the usual hyperpnœa.

Pupillo-motor reflex. The dilatation of the pupil produced by adrenalin might be due in part to central inhibition of the nucleus of the 3rd nerve, and this possibility was investigated. An anæsthetised cat was taken into a room with a dim light. The resting size of the pupil was approximately three-quarters of full dilatation and the pupil when illuminated by electrical torch diminished to one-third dilatation. The intravenous injection of 2 c.c. of .004 p.c. adrenalin dilated the pupil from a resting value of three-quarters to five-sixths of full dilatation. This adrenalin pupil when illuminated contracted rapidly from five-sixths to two-thirds dilatation. It is evident, therefore, that the pupil under the influence of adrenalin acts as effectively to the light stimulus as the pupil of the normal cat. The light stimulus acts through the nucleus of the 3rd nerve. Consequently the results prove that adrenalin does not diminish the activity of the 3rd nerve nucleus.

Conjunctival reflex. This reflex was tested by lightly touching the corner of the eye under the same conditions as those described for the pupillo-motor reflex. There was no evidence that it was diminished by the intravenous injection of adrenalin.

The absence of any demonstrable effect of adrenalin on the bulbar and mid brain centres in general, apart from the respiratory centre, offers strong evidence against the vaso-motor hypothesis of adrenalin apnoea. The general association both from an anatomical and physiological point of view, of the bulbar centres renders it very improbable that the blood vessels to one centre should be affected by a substance with so extensive an action as adrenalin, without the blood supply to the adjacent centres being affected.

Spinal reflexes.

The foregoing experiments show that the respiratory centre is the only cranial centre influenced by the intravenous injection of adrenalin. We therefore investigated the action of adrenalin on spinal reflexes associated with voluntary muscles, to determine whether adrenalin had an action on the cord similar to that observed with the respiratory centre.

Knee jerk. A record of the knee jerk was obtained by supporting the knee joint of a cat and attaching the foot to a writing lever. The patellar tendon was mechanically stimulated by light taps at 2 second intervals, and during this stimulation 1 c.c. of .02 p.c. adrenalin was intravenously injected. The record showed that the knee jerk was not influenced by adrenalin.

Crureus muscles. The reflex contractions of the left crureus muscles in a spinal cat were elicited by stimulating the central end of the ipsilateral sciatic nerve, the anterior crural (femoral) nerve being left intact. Tetanic stimuli lasting for 2 seconds repeated at 12 second intervals were used. During the regular series of reflex contractions 1 c.c. of .01 p.c. adrenalin was given intravenously. The tracing showed that the contractions were not effected.

The tone of muscles. A spinal cat was prepared and a record was obtained of the tone of the quadriceps extensor before, during and after the injection of a massive dose of adrenalin (.05 mgrm.). There was no indication of any change in the tone of the muscles during the general action of adrenalin in the animal. Similar results were obtained from an examination of the effect of adrenalin on the exaggerated postural tonus of the decerebrated cat.

*The antagonistic action of adrenalin and afferent nerve stimulation
on the respiratory centre.*

Afferent nerve stimulation produces an exaggerated discharge of the respiratory centre resulting in hyperpnœa. This hyperpnœa may be diminished or annulled by adrenalin. Fig. 5 shows the hyperpnœa produced by faradic stimulation of the central end of the right femoral

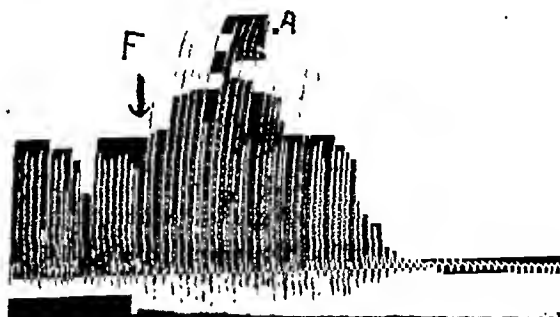


Fig. 5. Adrenalin on hyperpnœa produced by afferent nerve.
F, femoral nerve stimulated. A, adrenalin injected.

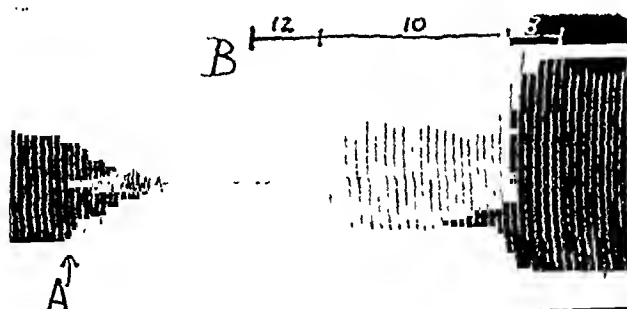


Fig. 6. Afferent nerve stimulation in adrenalin apnœa. A, adrenalin injected. B, femoral nerve stimulated with increasing strength of currents.

nerve and the marked diminution in respiratory movements produced during nerve stimulation by the intravenous injection, $\cdot 02$ mgrm. of adrenalin. The contrary effect may be demonstrated with equal certainty. Fig. 6 A shows the apnœa produced by $\cdot 01$ mgrm. of adrenalin. At B a faradic current was applied to the central end of the cut femoral nerve with the secondary coil at 12—no effect was produced; at B the secondary coil was moved up to 10 with a resultant return to normal respiration; at C the secondary coil was placed at 8 with a production of hyperpnœa.

DISCUSSION.

From the foregoing experiments it is evident that adrenalin effects the respiratory centre only and has no action on other bulbar centres or on parts of the brain concerned with the eye reflexes. These facts render the hypothesis of localised vaso-constrictor nerves to the cerebral vessels improbable. On the other hand, no evidence has been obtained from a study of muscle tone and limb reflexes that adrenalin has a direct action on the cells of the brain or the spinal cord. Therefore we must assume that adrenalin acts directly on the cells of the respiratory centre and apparently on the cells of the respiratory centre only. This hypothesis is supported by the mutual antagonistic actions of adrenalin and afferent nerve stimulation on the activity of the respiratory centre. Afferent nerve hyperpnoea may be diminished by adrenalin, or adrenalin apnoea may be annulled by afferent nerve stimulation. Since afferent nerve hyperpnoea is due to impulses affecting the cells of the respiratory centre it follows that the antagonistic action of adrenalin must be also due to an action of adrenalin on the same cells.

In a previous paper it has been indicated that the facts of adrenalin apnoea form an apparent contradiction to the generalisation that adrenalin acts as a defence mechanism in the body. The mutual antagonistic action of adrenalin and afferent nerve stimuli on the respiratory mechanism clears up this apparent difficulty. On the occurrence of danger afferent impulses tend to discharge the respiratory centre, and the resultant hyperpnoea, if not controlled would lead to a depletion of CO_2 from the blood with a production of asphyxia. But the simultaneous output of adrenalin diminishes the sensitivity to these stimuli and hyperpnoea is produced only when the CO_2 of the blood has accumulated to a sufficient degree to overcome this diminished sensitivity.

The facts of adrenalin apnoea does not favour the hypothesis of Lumsden (4) that respiration is controlled by four distinct centres. These centres of different function, localisation and development should be effected in varying degrees by adrenalin and corresponding variations in respiration be produced. In point of fact, adrenalin only diminishes to a greater or less degree the movements of respiration—inspiratory apnoea or gasping never being produced. This uniformity of adrenalin apnoea, and the absence of adrenalin action on any other nerve cell in the mid brain, bulb, or spinal cord indicate the existence of one centre only for the nervous control of the respiratory mechanism.

SUMMARY.

(1) Adrenalin apnoea is not accompanied by any change in the activity of the cardio-inhibitory centre, the vaso-motor centre or the swallowing centre. It is improbable, therefore, that adrenalin produces local vaso-constriction of the blood vessels to the bulb.

(2) Adrenalin does not influence the normal tone of muscles, the exaggerated tonus of decerebrate rigidity, the pupillo-motor reflex, the conjunctival reflex, or the reflex movements of the limbs.

(3) These experiments indicate that adrenalin effects respiratory movements by a specific action on the cells of the respiratory centre.

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- (2) Boruttau. *Pflüger's Arch.* p. 78. 115. 1899.
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- (4) Lumsden. *This Journ.* 57. p. 354. 1923.

Errata. Nos. 2 and 3, Vol. 59.

- p. 161. The Figure inscribed 'coil 6 cm.' is Fig. 5 (*not* Fig. 4), and that inscribed 'coil 6.75' is Fig. 4 (*not* Fig. 5) in accordance with the description in the text.
- p. 248, line 20 from top. For '·1 to 2 mg.' read '·1 to ·2 mg.'

THE DISSOCIATION OF OXYHÆMOGLOBIN IN THE TISSUES. BY J. ARGYLL CAMPBELL.

(From the National Institute for Medical Research, Hampstead.)

BOHR, HASSELBALCH and KROGH(1) discovered that the dissociation curve of hæmoglobin is greatly affected by the partial pressure of CO_2 present, the CO_2 helping to expel O_2 from its combination. Barcroft and Orbeli(2) showed that lactic acid has a similar effect, so that CO_2 produces its effect through its action as an acid. Barcroft and Camis(3) and Barcroft(4) demonstrated that alkalies have the opposite effect, rendering the oxyhæmoglobin more stable.

From these results it has been deduced that in severe exercise the increase of the H-ion concentration consequent on the formation of lactic acid causes an easier liberation of O_2 from Hb and thus to some extent counterbalances the decreased O_2 -saturation of the Hb. It seemed possible that the effect thus deduced might be demonstrated in a living animal by injecting gas under the skin and into the abdominal cavity and observing the changes which severe muscular exercise produces in the O_2 -tensions of the gas.

Method. The technique has already been fully described(5). Nitrogen was injected in moderate amount, under the skin of the back and into the abdominal cavity of a rabbit and left there two or three days until the CO_2 - and O_2 -tensions of the gas had come into equilibrium with the tensions in the surrounding tissue spaces. Then about 10 c.c. were withdrawn at intervals during the experiments without anaesthesia and analysed in the Haldane apparatus. The needles used to withdraw samples of gas from the abdominal cavity and from under the skin were about two inches in length, those for the abdominal cavity had an external diameter of .025 inch, those for the skin being wider, .035 inch in external diameter. The air space of these needles was reduced to a minimum and stilettes were used for the abdominal cavity.

The animal was excited to make movements of trying to escape by taking hold of one, or two, of its legs once every five seconds or so. Most of its body muscles were thus exercised. Some animals, less tame than others, performed more strenuous exercise. Obvious hyperpnoea

was produced in all cases but only in one or two special experiments was severe exercise performed.

I have injected O_2 and N_2 under the skin of the back of my own forearm, no pain being felt unless the arm was moved much. Then there was a slight gnawing pain here and there owing to the squeezing of the gas through the tissue. My skin was much tighter than that of a rabbit.

The effects of muscular exercise upon CO_2 -tensions in injected gas in rabbits. I have already demonstrated(5) that the CO_2 -tensions in gases injected under the skin and into the abdominal cavity of the resting rabbit were similar to one another and to that in the animal's alveolar air.

Table I gives results obtained from a rabbit with gas under its skin and in its abdominal cavity. The animal was given three periods of exercise separated by rest periods.

TABLE I. *Rabbit; 3.5 kilo.*

Time (mins.)	Tensions under skin. mm. Hg		Tensions in abdominal cavity. mm. Hg	
	CO_2	O_2	CO_2	O_2
0	47	26	47	39
4-11 exercise	—	—	—	—
12	52	23	57	36
22	54	23	55	36
32	53	23	49	33
35-42 exercise	—	—	—	—
43	54	22	50	35
53	51	22	44	39
99	41	24	40	39
110-120 exercise	—	—	—	—
121	50	23	54	34
181	39	25	36	42
228	39	26	38	44
288	40	28	38	46
356	39	29	37	47
23 hours later	44	30	44	39

It will be seen that the changes, produced by exercise, in the CO_2 -tensions in gas under the skin and in the abdominal cavity of the rabbit were similar to the course of events found by Douglas and Haldane(6) to occur in the alveolar CO_2 -tension in man, viz. that after exercise there is first an increasing CO_2 -tension then a fall below normal level and then a gradual return to normal; and that after each successive exercise period the rise is less than in the preceding period. Reference to the experiment, with their subject F.A.H., on page 433 of their paper will show great similarity of detail.

Twenty experiments of a similar nature gave constant results. Usually the CO_2 -tension changes under the skin lagged behind those in the abdominal cavity probably because of the less free circulation in the

former situation. Because of the obvious differences in experimental conditions it is difficult to compare the results for alveolar CO_2 -tension in man with those in injected gas in rabbits in greater detail. However, an examination of the results will show that the changes passed off more quickly in man than in the rabbit. This may have been due in part to the fact that the CO_2 -tensions in rabbits represented the conditions in the tissue spaces whereas in man the tensions were those for the alveolar air or arterial blood. The changes in the one must follow those in the other, but probably there is a lag in the changes in the tissue spaces so that the effects are slower in appearing and in passing off in this situation than in the arterial blood.

I have carried out some experiments with exercise in rabbits to compare the changes in alveolar CO_2 -tension with those for CO_2 -tension in gas under the skin. I used the Higgins-Plesch method to estimate the alveolar CO_2 -tension. By means of a small mask and rubber bag the rabbit rebreathed about 50 c.c. of air for various periods, e.g. 10, 20, 30 and 40 seconds and a curve was drawn for the CO_2 -tensions therein. Where the curve first became flat the CO_2 -tension was read off and assumed to be that of the alveolar air. The great drawback was the length of time necessary to obtain one reading, since several samples, at least three, of the rebreathed air were required. The results obtained for changes in alveolar CO_2 -tension were similar to those obtained for CO_2 -tension in gas under the skin but the value of the former was usually lower, and there was a distinct lag under the skin. It will be seen from the tables that the CO_2 -tensions in the abdominal cavity following exercise were, on the whole, lower than those under the skin.

From the above it was concluded that the general agreement between the results with my method and those for alveolar air was established, and that the changes for O_2 -tensions would therefore also be reliable.

The effect of muscular exercise upon the O_2 -tensions in gases injected into rabbits. It will be observed in Table I that the O_2 -tension in gas under the skin, before the exercise, was 26 mm. Hg; that in the abdominal cavity being 39 mm. As the result of exercise the O_2 -tension at first fell 4 mm. Hg in both situations, probably because of the lessened circulation in these regions since more blood was required in the muscles. Eventually when the CO_2 -tension was low, the O_2 -tension increased to 8 mm. Hg above normal in gas in the abdominal cavity and to 3 mm. above normal in gas under the skin. The changes in O_2 -tensions were slow in making their appearance in this experiment. In other experiments the O_2 -tension rose more rapidly.

Details of two such experiments are given in Tables II and III.

TABLE II. *Rabbit*; 1.8 kilo.

Time (mins.)	Tensions under skin. mm. Hg		Tensions in abdominal cavity. mm. Hg	
	CO ₂	O ₂	CO ₂	O ₂
0	46	19	46	33
25-36 exercise	—	—	—	—
37	46	22	57	31
48	43	23	43	33
111	37	28	—	—
149	40	31	—	—

TABLE III. *Rabbit*; 1.8 kilo.

0	53	23	53	40
2-6 exercise	—	—	—	—
6-10 rest	—	—	—	—
10-14 exercise	—	—	—	—
14-18 rest	—	—	—	—
18-22 exercise	—	—	—	—
25	59	22	—	—
95	39	29	39	51

In Table II it will be observed that the O₂-tension in gas under the skin was increased after the cessation of the exercise reaching 9 mm. Hg above normal in 75 mins. and 12 mm. above normal in 113 mins.; this indicated a rise of about 60 p.c. in O₂-tension. There was only a small amount of gas present in the abdominal cavity so that it was not possible to follow the O₂-tension changes in full.

Table III gives details of an experiment in which in about 73 mins. after the exercise the O₂-tension under the skin rose from 23 to 29 mm. Hg, whilst that in the abdominal cavity increased from 40 to 51 mm. Hg; these figures indicated a general rise in O₂-tension throughout the tissue spaces of about 25 p.c.

The presence of the gas under the skin and in the abdominal cavity cannot be regarded as a normal condition but that its presence has a negligible effect upon the results followed from the fact that the results were independent of the volume of gas present within the wide limits of 50 to 750 c.c. under the skin and of 50 to 350 c.c. in the abdominal cavity. Table IV illustrates this point and also that the results were independent of the absolute value of the O₂-tension.

It will be seen that notwithstanding the very different amounts of gas in the two parts of the experiment, the effects of muscular exercise were similar. In both there was a rise of CO₂-tension followed by a fall, and the usual definite rise of O₂-tension when the CO₂-tension was low. The volume of gas present in the animal was estimated from the total volume withdrawn in the samples.

TABLE IV. *Rabbit; 3.7 kilo.*

500 c.c. N_2 injected 65 hours previously under skin and 350 c.c. into abdominal cavity.
Gas remaining under skin about 300 c.c. and in abdominal cavity about 200 c.c.

Time (mins.)	Tensions under skin.		Tensions in abdominal cavity.	
	mm. H_2		mm. H_2	
	CO_2	O_2	CO_2	O_2
0	48	35	49	12
5-15 exercise	—	—	—	—
17	52	35	50	41
29	49	36	—	—
84	10	40	41	41
331	42	12	43	45
0*	51	25	51	39
1-6 exercise	—	—	—	—
7	57	25	—	—
9-14 exercise	—	—	—	—
16	66	23	—	—
27	66	23	38	41
32-37 exercise	—	—	—	—
40	64	24	10	44
53	53	26	35	46
103	35	33	37	48
165	11	32	11	48

* Two days later, gas remaining under skin about 50 c.c., and in abdominal cavity, about 60 c.c.

The rise of O_2 -tension was quite independent of the absolute value of the O_2 -tension before the exercise. In the first part of the experiment (Table IV) the O_2 -tension under the skin before the exercise was 35 mm. Hg that is 10 mm. higher than the O_2 -tension before the second part of the experiment; yet in both there was a rise of 7 mm. Hg in O_2 -tension after the exercise. The O_2 -tension after the first injection was higher than normal owing to local hyperæmia which, as usual(5), was present for the first two or three days after the injection of gas. Three experiments were carried out when this hyperæmia was at its maximum, that is when the O_2 -tension was 60 mm. Hg under the skin; the result of muscular exercise being that the O_2 -tension was increased by 9 mm. Hg (see Table V) the effect of lactic acid was thus superimposed upon that of hyperæmia.

TABLE V. *Rabbit; 3 kilo.*

Time (mins.)	Tensions under skin (mm. Hg)	
	CO_2	O_2
0	11	61
5-9 exercise	9-13 rest	13-17 exercise
17-21 rest	21-25 exercise	
115	31	69
155	31	70
220	38	68
350	38	68
19 hours later	18	39

The rise of O_2 -tension occurred only when there was a low CO_2 -tension due to lactic acid; of course, a low CO_2 -tension of the degree observed would in itself hardly affect the O_2 -tension. The rise of O_2 -tension was general, *i.e.* in the abdominal cavity as well as under the skin; it could not have been due to dilatation of blood vessels, since a general fall of blood-pressure produced a fall of O_2 -tension, *e.g.* after histamine (5).

The rise in O_2 -tension is evidently due to lactic acid causing an increased liberation of O_2 from Hb. It was demonstrated also following insulin convulsions. Some time ago (7) it was shown that subcutaneous injection of insulin in excessive doses and in doses insufficient to cause convulsions produced a rise of CO_2 -tension in gas under the skin of a rabbit and a fall of O_2 -tension therein; in some experiments, but not in all, similar results have been obtained in gas in the abdominal cavity. The curve of O_2 -tension appeared to follow the blood sugar curve fairly closely and seemed to be independent of circulatory changes. The above changes preceded the convulsions, the insulin itself appearing to lower in some way the O_2 -tension in the tissue spaces. After the convulsions the same changes for CO_2 - and O_2 -tensions as already described for muscular exercise, were produced; that is the CO_2 -tension was at first temporarily increased and then markedly decreased obviously owing to lactic acid formation. At the same time the O_2 -tension which had been lowered by the insulin increased and returned towards normal, the result being quite independent of the absolute value of O_2 -tension.

In the experiment illustrated in Fig. 1, the rabbit (4 kilos), which was not starved, was given an hourly dose of insulin. It will be seen that CO_2 -tension in gas under the skin rose slightly at first and then returned to normal, whilst the O_2 -tension fell gradually from 20 mm. Hg to 9 mm. following fairly closely the blood sugar curve; the blood sugar—estimated by Dr H. W. Dudley—fell from 140 mg. p.c. to 40 mg. p.c. About 50 minutes after the fifth injection of insulin, convulsions were produced and then again about 90 minutes later. Some time after the convulsions, the CO_2 -tension had fallen from 56 to 34 mm. Hg, whilst the O_2 -tension had risen from 9 mm. Hg to normal, *i.e.* 20 mm. No sample of gas was withdrawn immediately after the convulsions so that the expected temporary rise of CO_2 -tension was not observed in this experiment, although in others a rise of 11 mm. Hg was obtained. The rabbit, in the experiment described, completely recovered after an injection of glucose.

The rise of O_2 -tension produced by severe muscular exercise usually

passed off in an hour or two after reaching its maximum. In one or two cases the O_2 -tension remained a few millimetres above normal for

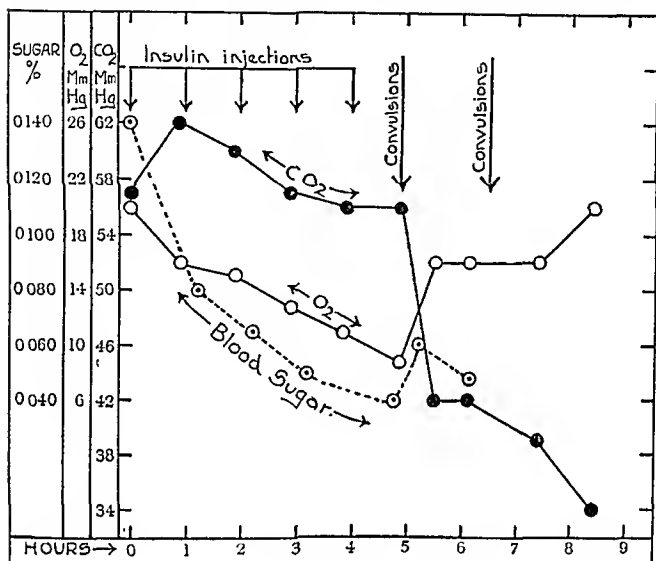


Fig. 1.

as much as 24 hours. The increase of O_2 -tension throughout the tissue spaces may possibly be concerned with the exhilarating after-effects of muscular exercise.

I have pointed out (5) that a rise of O_2 -tension above normal often followed a marked constriction produced by adrenalin or pituitary extract. The immediate effect of such constriction was an increase of CO_2 -tension in injected gas and a fall of O_2 -tension; in some cases when the effect had passed off the O_2 -tension not only returned to normal, but increased by 8 mm. above normal whilst the CO_2 -tension fell below normal. This may have been due to a local dilatation following the constriction, but was more likely due to production of lactic acid during the marked constriction so that the effect of lactic acid upon HbO_2 resulted.

Exercise to produce distress. High figures for CO_2 -tension in injected gases were obtained after exercise producing dyspnoea probably because of marked failure of the respiratory and circulatory mechanisms. Thus in one experiment the CO_2 -tension rose from 42 to 76 mm. Hg, and in another from 56 to 85 mm. Hg. Such high figures led me to suggest that high CO_2 -tensions in the muscles underlying the skin might directly influence the CO_2 -tension in the tissue spaces of the skin outside the muscle sheath. I also suggested (5) that the low O_2 -tension in resting muscle might lower the O_2 -tension in the overlying tissue spaces of the skin. I must abandon these suggestions since all the changes in gas tensions described above were general, that is, in the abdominal cavity as well as under the skin, and were obviously due to presence of acid substances in the circulating blood, so that the composition of the blood in muscular exercise determined the CO_2 -tension changes in the injected gases. That gases did not diffuse from one tissue to another was proved further since the CO_2 -tension in the gas contents of the gut was 78 mm. Hg whilst that in gas in the abdominal cavity was 42 mm., the O_2 -tensions being 10 and 42 mm. Hg respectively.

It has been pointed out that the O_2 -tension under the skin was always about 10 mm. lower than that in the abdominal cavity. All the evidence indicated that this was due to the better circulation in the abdominal cavity. Experimental changes in gas under the skin usually lagged behind those in the abdominal cavity; this was most likely due to the poorer circulation in the former region.

The effect of experimental alkalosis on injected gases in the living animal. As already stated Barcroft and his co-workers proved that alkalies rendered oxyhæmoglobin a more stable combination. Therefore it might be expected that the O_2 -tension in gas injected into the abdominal cavity and under the skin of a rabbit would be lowered by experimental alkalosis. There are several methods by which an experimental alkalosis may be produced, but other effects besides alkalosis occur which might also be held responsible for changes in gas tensions.

The effect of hyperpnoea produced by vigorous artificial respiration was first studied. I have already published (8) some results dealing with changes in CO_2 -tensions. I give herein details of changes in O_2 -tensions, both in gas under the skin and in the abdominal cavity. Schuster's (9) double action pump was employed and a cannula was inserted into the trachea of a urethanised rabbit. Table VI gives details of an experiment.

TABLE VI. *Rabbit; 3.5 kilo; urethane.*

Time (mins.)	Tensions under skin. mm. Hg		Tensions in abdominal cavity. mm. Hg	
	CO ₂	O ₂	CO ₂	O ₂
0	55	19	52	37
1 A.R. (commence)	—	—	—	—
23	50	18	28	35
53	48	17	18	33
60	45	16	11	35
94 A.R. (stop)	36	15	8	35
179	42	15	42	35
194 A.R. (commence)	—	—	—	—
230	45	9	13	31
259 A.R. (stop)	37	9	11	31
299	39	11	31	33

It will be observed that artificial respiration (A.R.) reduced the CO₂-tension both under the skin and in the abdominal cavity, but far more markedly in the latter region; under the skin it fell from 55 to 36 mm. Hg, whereas in the abdominal cavity it fell from 52 to 8 mm. Hg. These results agree with my published results(8); in the latter I showed that the O₂-consumption was increased by artificial respiration, the body attempting to replace the CO₂ pumped out. In the experiment in Table VI, 70 c.c. air was pumped into and withdrawn from the lungs 180 times a minute, so that the fall of CO₂-tension under the skin was remarkably slow, seeing that the pumping was continued for two periods of 93 and 65 minutes respectively.

It will also be observed that the O₂-tension fell in both regions, that is from 19 to 9 mm. Hg under the skin and from 37 to 31 mm. Hg in the abdominal cavity. Four experiments were carried out with similar results, the fall in O₂-tension being as much as 14 mm. in one experiment. After cessation of the artificial respiration, normal breathing returned and the animal's condition appeared to be excellent. It was obvious then that excessive hyperpnœa lowered the O₂-tension throughout the body, but we cannot conclude that this was due to alkalosis alone since artificial respiration also lowered the blood-pressure. Dale and Evans(10) found that a marked fall of blood-pressure was produced in cats and a less striking fall in rabbits by pumping out CO₂. I(8) have published similar results for blood-pressure. The anæsthetic, urethane, brings in another factor. I found(5) that urethane lowered the O₂-tension, but the rate and degree of decrease due to urethane were much less than those due to excessive hyperpnœa. Hill and Flack(11) were the first to show that in very vigorous hyperpnœa in man the alveolar CO₂-tension may be reduced to 10 mm. Hg, when breathing O₂, which lessened the discomfort of forced breathing. In the experiment in

Table VI, a similar low figure, *i.e.* 8 mm. Hg was obtained for CO_2 -tension in gas in the abdominal cavity of the rabbit during excessive artificial respiration.

In my next experiments I tried the effect of injecting 8 p.c. NaHCO_3 in saline solution into an ear vein of a rabbit without anæsthesia. Details of an experiment are given in Table VII.

TABLE VII. *Rabbit; 3.5 kilo; no anæsthetic.*

Time (mins.)	Tensions under skin.		Tensions in abdominal cavity.	
	mm. Hg		mm. Hg	
	CO_2	O_2	CO_2	O_2
0	51	30	51	43
17 (21 c.c. NaHCO_3 (8 %))	—	—	—	—
77	59	20	53	39
82 (12 c.c. NaHCO_3)	—	—	—	—
137	63	14	55	36
142 (20 c.c. NaHCO_3)	—	—	—	—
202	78	6	53	33
252	65	8	52	31
18 hours later	52	30	52	42

NaHCO_3 was injected very slowly in three separate injections amounting to 53 c.c., the results after each injection being similar. The CO_2 -tension under the skin was increased from 51 to 78 mm. Hg whilst that in gas in the abdominal cavity was increased from 51 to 55 mm. Hg. On the other hand the O_2 -tension was reduced from 30 to 6 mm. Hg under the skin and from 43 to 31 mm. Hg in the abdominal cavity. These results were to be expected from an alkalosis. Davies, Haldane and Kennaway⁽¹²⁾ showed that ingestion of NaHCO_3 raised the alveolar CO_2 -tension in man. Dale and Evans⁽¹⁰⁾ found that intravenous injection of NaHCO_3 (6–10 p.c.) produced greater alkalosis and a very much smaller fall of blood pressure than artificial respiration of the degree under consideration. Yet in my results the fall in O_2 -tension was much more marked after NaHCO_3 than during artificial respiration, so that the alkalosis due to NaHCO_3 and not the fall of blood-pressure was probably the chief factor in lowering the O_2 -tension in my experiments. My results for five experiments were similar although the quantities of NaHCO_3 injected varied from 10 c.c. 10 p.c. solution to 84 c.c. 8 p.c. solution. Signs of tetany were produced in three experiments but the animals seemed vigorous and not very uncomfortable; two of them were not destroyed immediately and the tensions returned to normal by next day (see Table VII). Greenwald⁽¹³⁾ states that tetany after NaHCO_3 is not due to alkalosis but to the Na -ion. I thought it would be of interest to study the effect of guanidin tetany upon gas tensions in injected gas in rabbits. Guanidin hydrochloride (0.2 to

0.3 gm. per kilo) was injected, dissolved in a small quantity of water, into an ear vein of a rabbit without anaesthesia. Table VIII gives details of an experiment.

TABLE VIII. *Rabbit; 1.55 kilo; no anaesthetic.*

Time (mins.)	Tensions under skin mm. Hg		Tensions in abdominal cavity. mm. Hg	
	CO ₂	O ₂	CO ₂	O ₂
0	48	26	48	34
15 guanidin HCl 3 g. per kilo	—	—	—	—
53	50	25	50	29
80	60	21	60	22
260	67	10	56	11
330	64	9	56	18

It will be observed that the effects of guanidin upon the CO₂- and O₂-tensions in the injected gas were similar to those already described for NaHCO₃. Tetany was produced but the animal was recovering and very active. Similar results were obtained in other experiments.

I have thus studied the effects of three conditions, excessive hyperpnoea, NaHCO₃ injections and injection of guanidin, all of which are known to cause tetany; the results for O₂-tension under the skin and in the abdominal cavity were similar in all three, a marked fall being produced. This fall of O₂-tension may or may not have been due entirely to alkalosis but was probably due in part to alkalosis. It is probable also that the tetany resulting from each of the three conditions is due to oxygen deficiency.

SUMMARY.

1. The changes produced, by muscular exercise, in CO₂-tensions in gas injected under the skin and into the abdominal cavity of rabbits are similar to those obtained for alveolar CO₂-tensions in man. Immediately after cessation of exercise there is a rise of CO₂-tension; this is followed by a fall due to the action of lactic acid upon the respiratory centre. Eventually the tensions return to normal.

2. Muscular exercise increases the O₂-tension in gas under the skin and in the abdominal cavity by 25 p.c. on an average. This rise is regarded as being due to the action of lactic acid upon the dissociation of HbO₂, which effect is thus demonstrated in the living animal. Insulin convulsions have a similar effect to muscular exercise.

3. Artificial respiration greatly reduces whilst intravenous injection of NaHCO₃ or of guanidin hydrochloride greatly increases the CO₂-tensions under the skin and in the abdominal cavity.

4. Artificial respiration, NaHCO_3 and guanidin greatly reduce the O_2 -tension in injected gas producing a marked oxygen deficiency in the tissue spaces, which it is suggested may be responsible for the resulting tetany. The fall of O_2 -tension may, in part, be due to alkalosis.

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ON THE FORMATION OF SULPHÆMOGLOBIN

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IN a paper on sulphæmoglobin, some time ago, Wood Clarke and Hurtley⁽¹⁾ arrived at the conclusion that reduction is an essential factor in the formation of sulphæmoglobin. They were led to this opinion by the following experiment: A certain quantity of fresh blood, diluted with water, was divided into three parts. One part was placed in a small cell (*a*). To the remaining two-thirds a few drops of sulphuretted hydrogen water were added, and half of this specimen was placed in a second cell (*b*), and the remainder in a third cell (*c*). To the fluid in each of the cells *a* and *b*, a drop of a 4 p.c. solution of phenyl-hydrazin was added whilst the liquid was stirred with a glass rod. The blood in cell *a*, which contained only blood and phenyl-hydrazin, showed reduction, but no trace of a sulphæmoglobin band. In the contents of cell *c*, which contained only blood and hydrogen sulphide, a faint sulphæmoglobin band was visible, after standing for a considerable time. The contents of cell *b*, on the other hand, showed an intense sulphæmoglobin band, even after a few seconds.

This experiment, which is easily performed, and is always successful provided that the different solutions are in the right proportions, shows that the addition of phenyl-hydrazin,—and the same is true for hydrazin—to a mixture of blood and sulphuretted hydrogen accelerates conspicuously the formation of sulphæmoglobin, but it does not prove that the acceleration is due to the reducing action of these substances, and still less that the formation of sulphæmoglobin is necessarily preceded by reduction. Clarke and Hurtley pointed out that their results tended to confirm Harnack's view⁽²⁾, that sulphæmoglobin is formed by the action of hydrogen sulphide upon reduced hæmoglobin, which as they pointed out is in contradiction to the older view of Hoppe Seyler⁽³⁾, who held that sulphæmoglobin cannot be formed from reduced hæmoglobin. Harnack attributed the supposed mistake of Hoppe Seyler to his use of carbon dioxide for the reduction of hæmoglobin. He found that carbon dioxide forms with oxyhæmoglobin a new compound which he called acid-hæmoglobin, with which hydrogen sulphide

does not form sulphæmoglobin, and assumed that Hoppe Seyler mistook acid hæmoglobin for reduced hæmoglobin.

In the course of our clinical studies of sulphæmoglobinaemia the question arose whether the generalization of Clarke and Hurlley, who ascribed the influence of hydrazin and phenyl-hydrazin upon the formation of sulphæmoglobin to a reducing action, was justified. We accordingly tried to ascertain whether other reducing substances, when added to a mixture of blood and hydrogen sulphide accelerated, in the same way, the formation of sulphæmoglobin. A certain quantity of fresh ox-blood, or even human blood, was beaten up and diluted with water until the hæmoglobin content of the solution equalled approximately $2\frac{1}{2}$ p.c. (determined according to Sahli). To 8 c.c. of this fluid we added 1 c.c. of a 0.06 p.c. aqueous solution of H_2S . The solution so obtained was divided into two parts. To one part we added 1 c.c. of the solution of the substance to be tested, and to the other in (the control) cell 1 c.c. of water.

It appeared that a number of substances have an accelerating action, but in very different degrees. The substances we examined were the following:

Phenyl-hydrazin: very intense; one drop of a 0.1 p.c. solution showed a distinct acceleration.

Hydrazin: intense.

Hydroxylamine: intense.

A positive result was also obtained with alpha-naphthylamine, diphenylamine, para-phenylene-diamine, dimethyl-paraphenylene-diamine, isatin, para-amidoacetophenone, dimethyl-amido-azobenzol, vanillin, aniline oil.

The action of phenyl-hydrazin far surpasses that of the other substances.

A negative result was obtained with resorcin, nitrobenzol, alpha-naphthol, cystin, urea, uric acid. This result lent no support to the view that the accelerating action of phenyl-hydrazin is due to its reducing power, for hydrazin reduces oxyhæmoglobin very quickly and strongly, much more quickly than phenyl-hydrazin, which exerts a complicated, and as yet unknown influence upon oxyhæmoglobin. If a small quantity of the reagent be added no reduction is observed; with a larger addition the band of reduced hæmoglobin appears indeed, and is replaced by the bands of oxyhæmoglobin on shaking with air, but at the same time the solution becomes turbid and assumes a brown colour, whilst the band becomes fainter and less sharply defined. There can be no doubt that the hæmoglobin molecule undergoes decomposition, and that as far as hydrazin and phenyl-hydrazin are concerned, there is no parallelism

between reducing action and acceleration of sulphæmoglobin formation. Moreover, resorcin, which is a powerful reducing agent, causes no acceleration. Lastly it must not be forgotten that phenyl-hydrazin is a very active substance, and acts not only as a reducing, but also as an oxidising agent.

In order to test the matter under the most simple conditions, we proceeded to bring about the reduction of oxyhæmoglobin by two methods, neither of which involved the use of any powerful chemical reagent: (1) by passing hydrogen through the solution for a considerable time, and (2) by "pumping out" the blood by means of a vacuum pump. The results of our experiments left no room whatever for doubt that: (a) sulphuretted hydrogen has not the slightest action upon reduced hæmoglobin, and brings about no formation of sulphæmoglobin therewith; (b) traces of oxygen suffice to bring about the formation of sulphæmoglobin in mixtures of sulphuretted hydrogen and hæmoglobin solution.

These results are in full accord with the earlier view of Hoppe Seyler. We have been able to explain Harnack's result, for the quantities of oxygen required to bring about the formation of sulphæmoglobin are so minute that a very complete removal of oxygen is necessary to prevent the change, and the removal of the last traces is a matter of no little difficulty. We are not justified in concluding as Harnack seems to have done (p. 575) that when both oxyhæmoglobin bands have disappeared entirely, reduction is complete. Only after hydrogen has been passed through the liquid for a long period, or after the blood has been "pumped out" slowly and with due precautions, is complete reduction obtained.

In such experiments it has usually been thought to be sufficient to cover the surface of the liquid with paraffin after pumping out, and so to exclude oxygen. But paraffin allows the passage of oxygen, although very slowly, and this method is quite inadequate (Eykmán, oral communication). Nor must it be forgotten that rubber tubing allows the passage of traces of oxygen. We therefore adopted the following methods:

(1) *Reduction by hydrogen.* The gas formed in a Kipp's apparatus was passed through an alkaline solution of pyrogallol, and afterwards through the solution of hæmoglobin. This solution was contained in a small glass apparatus which excluded any possibility of entrance of air. Of several types of apparatus tried that represented in Fig. 1 proved the most satisfactory.

For a hæmoglobin solution corresponding to Sahli = 1, it was found to be desirable to let the hydrogen bubble slowly through the solution for several hours. It must be remembered that a solution of hydrogen sulphide in water is only free from oxygen after air has been excluded for a considerable time, such as a few days. Concentrated solution must be employed, but of course in small quantities. Before commencing the experiment the stopper was removed from the tube, and the pipette with a stopcock was sucked full of hydrogen sulphide solution. The cock was closed, the stopper replaced on the tube, and the apparatus was then ready for the passage of the hydrogen through the liquid. When the reduction was considered to be complete, the supply of hydrogen was turned off, and the stopcock in the pipette was opened. The watery solution of hydrogen sulphide then ran into the tube, and the hydrogen displaced escaped through the outlet tube. When the level of the hydrogen sulphide water had fallen to just above the tap, both tap and outlet were closed. To prevent the entrance of air through the outlet tube, it was found to be desirable to make the outlet tube of considerable length, and to keep its orifice depressed.

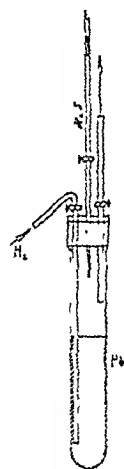


Fig. 1.

After the passage of hydrogen had continued for several hours the two bands of oxyhæmoglobin were replaced by the single band of reduced hæmoglobin, but on adding hydrogen sulphide sulphhæmoglobin was still formed, although only very slowly and in small amount. After the gas had passed for another hour the spectroscope showed no formation of sulphhæmoglobin, even though the liquid was allowed to stand for a week. If on the other hand air was admitted above the mixture sulphhæmoglobin was immediately formed.

As it seemed possible that a compound of hydrogen sulphide and reduced hæmoglobin was formed, but which did not yield the sulphhæmoglobin spectrum, the following experiment was tried. After the addition of hydrogen sulphide to the completely reduced hæmoglobin, hydrogen was again passed through the liquid to expel the hydrogen sulphide. On subsequent admission of air no sulphhæmoglobin was formed, and it was evident that the hydrogen sulphide had been completely removed by the passage of hydrogen, and that the hæmoglobin and hydrogen sulphide were not in combination, or at any rate that no stable compound was formed. It is not possible to expel hydrogen sulphide from a solution of sulphhæmoglobin in this or any similar way.

(2) We also brought about *reduction* by means of a *vacuum pump*. The solution of hæmoglobin was heated to a temperature of 37° , and a water suetion pump was employed. The liquid boiled and the oxygen was driven out with the vapour. The tube employed had an inlet and an outlet only, and the inlet was connected with a bottle containing hydrogen sulphide in aqueous solution. Before beginning the experiment the air in the inlet tube was ontirely driven out by hydrogen, and the vaeuum chamber was filled with hydrogen. After the reduction was completed a small quantity of sulphuretted hydrogen solution was sucked into the tube, and all the openiugs were closed. This method is less satisfactory than the former one, as a portion of the fluid is apt to be lost, owing to frothing during boiling. However, the same results were obtained whichever unethod was employed.

It having been ascertained that in the absence of oxygen no formation of sulphæmoglobin occurs, it was necessary to ascertain whether addition of phenyl-hydrazin to a similar mixture of hydrogen sulphide and rduced hæmoglobin caused formation of sulphæmoglobin. This was shown not to be the case.

How, then, was the accelerator action of phenyl-hydrazin to be explained? The simplest explanation appeared to be that phenyl-hydrazin causes the molecular oxygen of the air to combine with the sulphuretted hydrogen, thus giving to the sulphur or hydrogen sulphide an opportunity of combining with the hæmoglobin molecules. Some support for such an explanation is supplied by the fact that when a solution of phenyl-hydrazin is added to one of hydrogen sulphide, a turbidity appears after a few minutes. It is highly probable that this turbidity is due to liberated sulphur, but we have not succeeded in proving with certainty that such is the case. It is noteworthy, moreover, that if the same experiment be carried out with all precautions to exclude air, no turbidity is developed. Hence it seems probable that here, as in the preceding experiments, the phenyl-hydrazin exerts an activating influence upon the oxygen. It must be mentioned that in order to produce turbidity in a mixture of phenyl-hydrazin and hydrogen sulphide solutions in the presence of air, much larger quantities of phenyl-hydrazin are required than when blood is present in the solution.

SUMMARY.

1. Hydrogen sulphide combines with hæmoglobin or reduced hæmoglobin only in the presence of oxygen. It has no effect upon reduced hæmoglobin if air be completely excluded.

2. The accelerating action of phenyl-hydrazin upon the formation of sulphæmoglobin from hydrogen sulphide and hæmoglobin is only observed if oxygen be present. Mere traces of oxygen suffice.

3. Phenyl-hydrazin quickly produces a turbidity when added to a solution of hydrogen sulphide, provided that air be present. The turbidity is probably due to liberation of sulphur.

4. In all such experiments the phenyl-hydrazin appears to act as an activator of oxygen.

The fact, which Hoppe Seyler observed, that as far as the action upon it of hydrogen is concerned, hæmoglobin behaves as a much more stable substance than oxyhæmoglobin, appears to us to be of some biological importance. Hoppe Seyler(4) also found that reduced hæmoglobin is far the more resistant to the action of trypsin and to processes of decomposition. Lastly, it would appear that the formation of acid hæmoglobin under the influence of carbon dioxide, does not take place if oxygen be wholly excluded.

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THE INFLUENCE OF CHEMICAL FACTORS ON THE CORONARY CIRCULATION. BY R. HILTON (*Beit Memorial Research Fellow*) AND F. EICHHOLTZ (*Rockefeller Travelling Fellow*).

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THE previous experiments made in this laboratory^(1, 2) and elsewhere, led to the conclusion that, *in the heart isolated from the rest of the body*, one of the main factors influencing the coronary circulation was the formation of metabolites by the heart muscle itself. Nakagawa found that the coronary flow is practically uninfluenced by fairly wide ranges of temperature, by alterations in the rate of the heart-beat brought about by excitation of the sino-auricular node, by stimulation of the vagus nerve, or by alterations in the inflow and consequent output of the heart. In all these cases large variations are produced in the metabolism of the heart as measured by its oxygen consumption (Evans⁽³⁾). If the products of the metabolism of the heart muscle played an important part in adjusting the calibre of the blood vessels to the needs of the heart for oxygen, we would expect that in all these cases of increased metabolism there would also be increased production of metabolites and dilatation of the coronary vessels, which is not the case. In fact, under these conditions there is a lack of adaptation of the blood supply of the heart to its needs, a condition which we can hardly expect to hold in the intact animal, where we may expect further research to reveal other means of adapting the coronary flow to the needs of the heart.

The chief evidence for the importance of metabolites in regulating the blood flow through the organ was from the perfusion experiments of Barcroft and Dixon⁽⁴⁾, also the observation of Morawitz on the whole animal, that the flow increased on injecting into the circulation blood which had already flowed out from the coronary sinus, and the observation of Markwalder and Starling, that the maximum flow through the coronary vessels occurred in asphyxia just before the heart failed altogether¹.

¹ This result was considered by Markwalder and myself to be due to the formation of non-volatile metabolites. Later consideration, however, convinced me that the conclusion was not warranted, since the direct effect of O₂-lack on the coronary vessels was not investigated. E. H. Starling.

In the following pages we shall show that this evidence is illusory except so far as a moderate effect of CO_2 and lactic acid is concerned.

Methods. All the experiments were carried out on dogs of medium size anæsthetised with chloralose 0.1 gm. per kilo, after a preliminary small dose of morphia. A heart-lung preparation was made in the ordinary way and a Morawitz cannula introduced through the right auricular appendage into the coronary sinus. By this means, as shown by Evans and Starling(5), it is possible to collect about three-fifths of the total blood passing through the coronary vessels, the other two-fifths escaping into the right heart through the posterior cardiac veins and the veins of Thebesius. This ratio varies slightly at different rates of flow and this variation must be borne in mind when considering the value of the calculations of the oxygen consumption of the blood. The clip on the inflow from the venous reservoir was adjusted so as to give a total systemic output of between 300 and 400 c.c. per minute. The arterial resistance was adjusted to give a mean arterial pressure in the aorta of 90 to 100 mm. Hg. The temperature was kept as steady as possible at about 36°C . The coronary flow was measured by receiving the blood into graduated vessels and determining the outflow during 15 seconds. Samples of arterial and of coronary vein blood were taken at intervals in tubes under paraffin, and were examined the same day in order to determine hæmoglobin, hydrogen ion concentration (Dale-Evans method), content in CO_2 , and saturation in oxygen. Artificial respiration was maintained by a Schuster pump. By this pump it was possible to administer at any time either pure nitrogen or mixtures of nitrogen with air, of CO_2 with air, or of air with oxygen, the appropriate mixtures being prepared before the experiment and stored in rubber bags.

The alleged kneading action of the heart. Since in our experiments our different procedures, such as the administration of carbon dioxide, or asphyxiation, might cause changes in the rhythm of the beat and in the state of dilatation of the heart cavities, it was important to make certain that these in themselves would not give alterations in the coronary flow brought about by purely mechanical means. According to Wiggers(6) an important part in the circulation through the coronary vessels is played by the kneading action of the heart muscle when it contracts. If this were so, any change in strength or rhythm of beat or in tone of the heart muscle, should be attended with corresponding changes in the coronary flow. We have already seen that this idea is not borne out by Nakagawa's results(2): these we have been able to confirm. Nakagawa increased the rate of the beat by stimulation of the sino-auricular node and diminished its rate by stimulation of the vagus: in neither case did he observe corresponding changes in the coronary circulation. Any such action is put out of court by the following experiment.

A heart-lung preparation having been made, the heart of another dog was exposed, a cannula placed in the pulmonary artery, and then a cannula, connected with the arterial side of the heart-lung preparation, was tied into the aorta. The whole heart was then rapidly cut out of the body, the superior and inferior venæ cavæ having been previously

ligatured, and the organ was suspended above a funnel which led into the reservoir of the heart-lung preparation. The circulation through the vessels of the excised heart was thus maintained by the heart-lung preparation. The coronary blood flowed into the right auricle and was then expelled by the right ventricle through the cannula in the pulmonary artery, its rate of flow being measured. The heart continued to beat vigorously. After the blood flow through the coronary vessels had been measured in this way for some time, the rhythmic contractions of the heart were abolished by putting the ventricles into fibrillation by means of a strong faradic current applied to the myocardium. The results are shown in the following table.

TABLE I. Effect of fibrillation on coronary flow. Heart perfused *via* aorta from heart lung preparation.

Time mins		Coronary flow c c. per min.	B P. mm Hg	Pulse	Arterial O ₂ saturation
	Air				
1		75	118	128	100 %
2		75		128	
3		75			
	Nitrogen				
4		90			
5		100			
6		125			
7		174	110		
8		220			37 %
	Air				
10		160			
11		131			
			118	120	
19		107			
21		107			
22		107			
23		107			
24		107			
	Fibrillation				
27		167	118		
29		125			
30		134			
	Nitrogen				
32		139			
33		167			
33 5		203			
34		227			
34 5		250	108		47 %
	Air				
35		230			
36		214			
37		203			
38		172			
40		152			
41		150			100 %

Two points are worthy of note. The first is that when the heart ceased to beat rhythmically, each of its fibres contracting independently and rapidly, but in such a way that it was impossible to imagine any kneading effect on the contents of the cardiac vessels, the circulation through the coronary system was increased. At the same time the oxygen consumption was also greater than before fibrillation.

When we examine the flow from the coronary sinus we notice that it pulsates with each beat of the heart. There is no doubt, therefore, that the flow through the coronary vessels is affected to a certain extent by the heart-beat, but we consider we are justified in excluding any such mechanical effects from our consideration of the results obtained by altering the gaseous contents of the blood.

The effect of changes in the oxygen saturation of the blood on the coronary circulation. The great increase in coronary flow observed during asphyxia has been ascribed to the action of metabolites produced presumably in the heart muscle during the asphyxia. These metabolites should therefore be absent from freshly shed blood which has been deoxygenated by mere

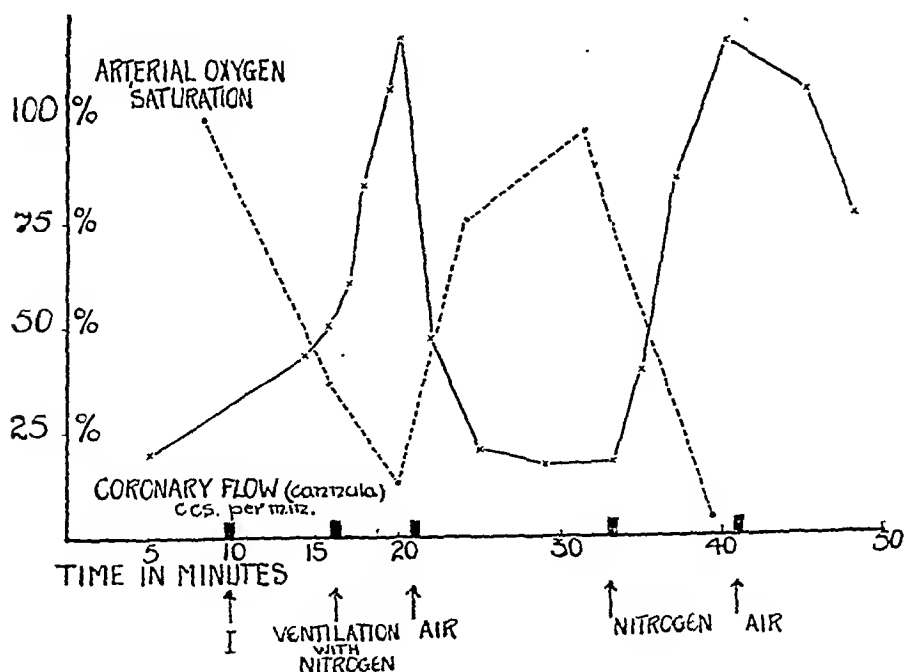


Fig. 1. Effect on the coronary flow (as measured by a cannula in the coronary sinus) of reducing the O_2 saturation in the arterial blood supply, with the b.p. kept constant (see text). At signal I the blood in the venous reservoir was replaced by deoxygenated blood which had not circulated through the preparation.

exposure to a vacuum. In the experiment of which the details are represented in Fig. 1, after the coronary flow had been observed to be steady at 20 c.c. per minute, the blood in the reservoir was run off and replaced by defibrinated blood which had been deprived of its oxygen by exposure to a vacuum, while the respiration pump was shut off. Although this blood took up a certain amount of oxygen on its way round the lungs before reaching the aorta and coronary arteries, it will be noticed that it produced an immediate rise in the coronary flow. After 7 minutes artificial respiration was started, using pure nitrogen for insufflating the lungs. This produced a further reduction of the blood and a sharp rise in the coronary flow, which increased to 120 c.c. per minute, five times that obtained previously. When the flow seemed to have attained its maximum and the oxygen saturation of the arterial blood had fallen to 12 p.c. the nitrogen bag was removed and respiration continued with air. It will be seen that there was at once a rise in the oxygen saturation of the blood and a fall in the coronary flow, which returned to normal about the same time that the oxygen saturation of the blood had been restored to 99 p.c. The experiment was repeated using only pure nitrogen. The oxygen saturation of the blood fell almost to zero, while the coronary circulation increased to the same extent as it had on the previous occasion.

In a second experiment the addition of previously deoxygenated blood was omitted and a rapid deoxygenation effected in the lungs themselves by using pure nitrogen for purposes of artificial respiration. An attempt was made to prolong the action and slow the recovery by using air diluted with a large amount of nitrogen. With the artificial respiration employed this was sufficient, however, to increase the oxygen saturation to 80 p.c., while the coronary circulation fell from 250 to 70 c.c. per minute, from which figure it only fell slightly on substituting pure air for respiration (Fig. 2).

In order to make a greater number of observations during the course of the experiment the deoxygenation of the blood was effected, using first nitrogen with a low percentage of oxygen before passing on to pure nitrogen (Fig. 3).

The results are seen to be the same as in the first experiment: a moderate rise of coronary flow as the oxygen saturation fell from 100 to 84 p.c., followed by a large rise on substituting pure nitrogen for insufflation, when the oxygen saturation fell to 25 p.c. and the coronary flow rose to 86 p.c., namely, three and a half times its resting value.

Oxygen consumption of the heart during anoxæmia. In all our experiments the oxygen saturation of the coronary blood was taken as

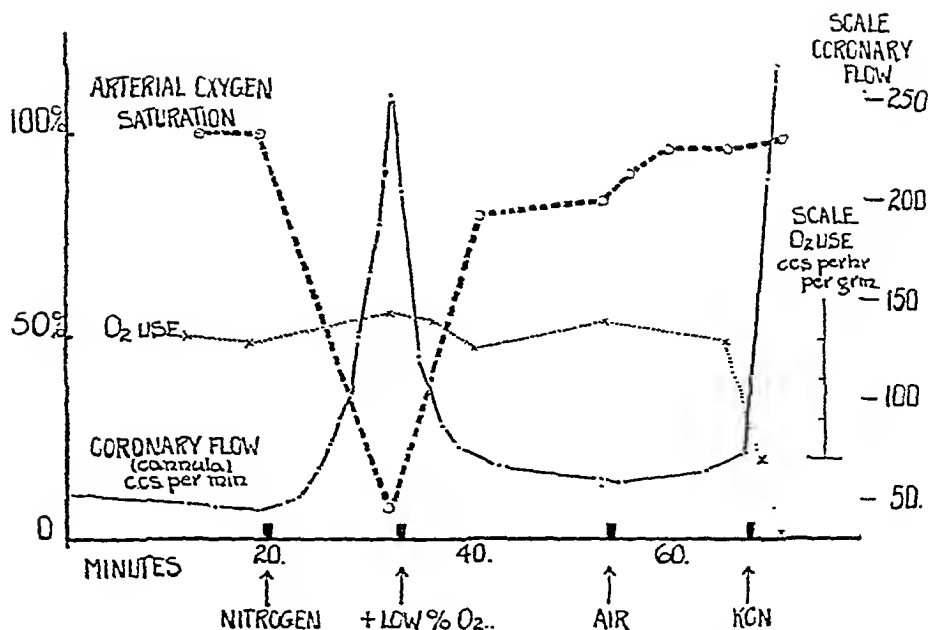


Fig. 2. Slowing of the recovery by ventilation with a mixture containing a low percentage of oxygen. The oxygen use seems to show no considerable variations, until KCN is added, when it falls to zero, the coronary flow rising to its maximum at the same time.

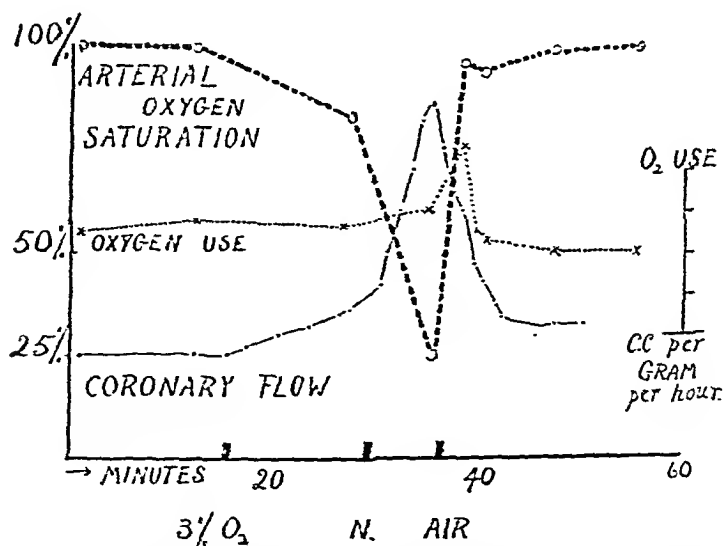


Fig. 3. Noticeable effect on the flow of even a slight fall in arterial oxygen saturation. The O_2 use, as measured, fails to show any oxygen lack. B.P. constant as before.

nearly simultaneously as possible with that of the arterial blood. Knowing the oxygen saturations of the arterial and venous bloods, as well as the rate of flow through the coronary vessels, we can calculate the oxygen consumption of the heart during the same period, and these results are given in the dotted line in the chart. Since the results obtained have to be multiplied by the rate of flow of the blood, they cannot claim to be free from slight errors depending on unavoidable differences in the periods during which the arterial and venous samples were taken, and the slight fall of pressure causing a slowing of the coronary flow which necessarily accompanied the taking of the arterial sample. It is satisfactory to note, however, that the figures we obtained for the oxygen consumption by the heart, namely, between 2 and 5 c.c. per gm. per hour, agree with those found by Evans as a result of the observations of the total gaseous exchanges of the heart during a given period. In Evans's first three protocols the oxygen consumption of the heart varied between 2.2 and 6 c.c. per gm. per hour (3).

If the regulation of the blood flow through the coronaries were effected by metabolites which accumulate as a result of oxygen lack, there should be evidence of a diminished consumption of oxygen by the heart muscle during the period of anoxæmia, provided its mechanical efficiency remains unaltered. In experiments of short duration there may be no evidence of any oxygen lack during the anoxæmia: the increased rate of flow keeps pace with and compensates for the diminished oxygen saturation of the blood. The heart can take oxygen out of the blood until this fluid is completely de-oxygenated, as shown previously by Evans and Starling. The dilatation, consequently, cannot be due to a lack of oxidation of metabolites; moreover, the immediate and considerable increase in the rate of coronary blood flow caused by decrease of oxygen tension in the blood cannot be imitated by the addition of CO_2 and lactic acid, even in quantities far in excess of those actually formed in such a sudden anoxæmia. The reaction of the arterial wall thus seems to be a direct one to the diminished oxygen tension of the circulating blood and is not indirectly due to asphyxia of the heart muscle. In a few cases there may be evidence of a short lasting oxygen debt, for the oxygen consumption during anoxæmia is often somewhat increased, and in these few cases remains so for a little time after the normal oxygen condition of the blood is restored. The fact that we obtain a maximal dilatation of the coronary vessels with complete anoxæmia, and a dilatation which is inversely proportional to the oxygen saturation of the blood, without any evidence of oxygen lack in the

contracting heart muscle or an oxygen debt incurred by the heart, removes any justification for ascribing these results to any metabolites accumulating in the heart muscle as a result of lack of oxygen.

Effect of cyanides. In the experiments quoted the deprivation of oxygen was brought about fairly rapidly or slowly by the use of nitrogen for artificial respiration. We have, however, a means of suddenly checking any intake of oxygen by animal tissues, namely, the use of hydrocyanic acid. We know that skeletal muscle can continue to function in the complete absence of oxygen or in the presence of HCN, and the same has been shown by Weizsäcker(8) to apply to the cardiac muscle of the frog. We have found that Weizsäcker's results can be applied also to the mammalian heart. When HCN is introduced into the blood circulating through the heart-lung so as to attain a concentration of $M/600$ in the blood, the heart continues to beat and to maintain a normal blood-pressure of 100 mm. Hg. for 3 to 5 minutes, and with a smaller dose— $M/3000$ —the heart has continued to beat for as long as 20 minutes and to maintain a normal arterial blood-pressure. Under these conditions, therefore, at any rate for a certain length of time, we can introduce cyanides or HCN into the blood circulating through the heart-lung apparatus without altering the chief mechanical condition, namely, the arterial blood-pressure, which determines the amount of the flow through the coronary arteries.

In striking contrast to this absence of immediate effect on the heart is the result of adding HCN to the blood on the coronary blood flow and on the oxygen usage by the heart. The coronary blood flow rises instantaneously to its maximum, generally about five times the value obtained at the beginning of the experiment, while the oxygen consumption by the heart as measured by the difference in saturation between arterial and venous bloods, falls to zero, the blood flowing through the coronary cannula being of a bright arterial colour. Here there can be no time for the production of metabolites: the only thing that we have altered is the possibility of taking up any oxygen by the tissues. This alteration affects only the recovery process of the heart muscle and therefore produces heart failure only after several minutes. The effect on the coronary blood vessels is, however, instantaneous, namely, complete relaxation, equivalent to that observed at the height of artificially induced anoxæmia (Figs. 2 and 4).

It is interesting to note that by the action of HCN we can distinguish two classes of tissues in the living body whose modes of action seem to be fundamentally different. In the first class, activity, in the ordinary

sense of the term, is anaerobic, the oxygen intake being concerned in and necessary for the processes of recovery only. To this class belong skeletal muscle, heart muscle, and probably salivary glands (if we may take the results of Bottazzi on the salivary glands of Octopus as applicable also in the higher animals). In the second class of tissues the intake of oxygen is essential to and contemporaneous with activity, which may be therefore regarded as continually aroused and kept up by the presence and intake of oxygen. To this class belong the kidney (Starling and Verney(7)), the unstriated muscles of the coronary arteries and probably of other arteries of the body. There is some evidence that the same is true for visceral unstriated muscle, but this requires further investigation (cp. Evans(9) and Weizsäcker(10)). If we regard the activity of the arterial muscle as directly due to the presence of oxygen and in a measure proportional to the oxygen tension or oxygen availability, we avoid the logical difficulty, which troubled Pflüger, of regarding a physiological result as occasioned by the *absence* of a substance. It was probably this logical difficulty which led to the assumption of the interaction of metabolites in the various phenomena produced by oxygen lack. But in the light of our experiments such an

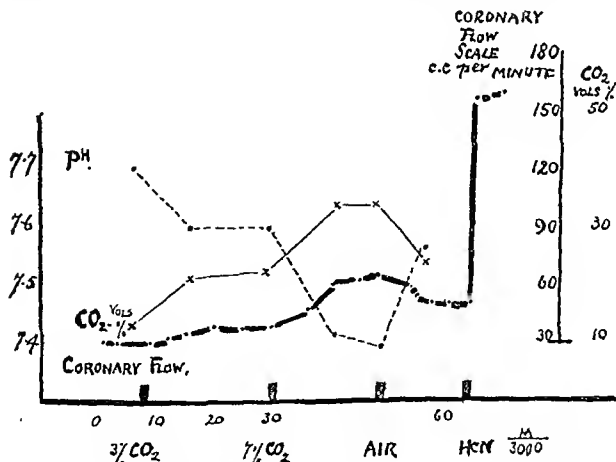


Fig. 4. Effect of ventilation with CO₂ on the coronary flow, and the accompanying pH changes. Also the immediate result of adding HCN to M/3000. B.P. constant.

assumption, at any rate as an explanation of the reaction of the coronary vessels to anoxæmia, becomes unnecessary and is therefore unjustifiable.

The influence of changes in the reaction of the blood on the coronary vessels. It was observed by Markwalder and Starling that dilatation of the coronary vessels and increased coronary flow was produced by the addition of CO_2 to the inspired air, though the effects were much slighter than those observed in the course of asphyxia. These results we have confirmed. The effect of altering the pH of the blood by the inhalation of CO_2 mixtures is given in the experiment represented in Fig. 4, and the effects of addition of lactic acid in Table II. It will be noted that the effects are quite small. In the experiment with lactic acid the change in pH was only minimal, so that the question arises whether we are dealing here with an effect of hydrogen ion concentration or with a specific result of the presence of excess of lactic ions themselves.

TABLE II. Effect of adding 100 mgm. % lactic acid on coronary blood-flow.

Time		Coronary flow c.c. per min. (3/5)	O ₂ saturation %	pH	Pulse	Temp.
1		40	100	7.8		35
4		44			138	
8		47				
11		50				
12	Lactic acid added	58				
13		64			118	
14		63				
15		66				35
15.5		65.6	100	7.7		
16	Fresh blood added	48				
18		58				
19		53				
20		58			140	
22		60				
23		60				
28		58			140	
30	Ventilation with nitrogen	64				
32		74				
33		84				
34		94	66	7.84		

The results in Table II show far greater increase of coronary flow with an arterial saturation of 66 p.c. than with the addition of 100 mgm. p.c. lactic acid; and this in spite of the fact that the blood-pressure at the end was 71 mm. Hg., whereas at the time of the addition of lactic acid it was 91 mm. Hg. The effect of a 20 mm. change in blood-pressure at this level is considerable.

The accumulation of metabolites in the blood. One of the reasons which led Morawitz to ascribe an important part in the dilatation of the coronary vessels to locally produced metabolites, was the fact that the flow of blood from the coronary sinus gradually increased in the course of the experiment as the coronary venous blood which had been drawn off was re-introduced into the circulation. In almost all our experiments we have also observed this same gradual increase in the coronary blood flow. In order to decide whether it was due to the accumulation of metabolites in the blood, we carried out the following experiment. After the usual preparations the coronary blood flow was measured until it became constant (the flow is usually quicker and the oxygen use also higher at the beginning of the experiment and then gradually slows, a condition of things which we have been accustomed to ascribe partly to the handling of the heart necessary for the introduction of the Morawitz cannula, and partly to the disappearance of adrenalin from the blood). Two-thirds of the blood in the venous reservoir was then drawn off and kept at body temperature in a warm bath. the rest of the blood was allowed to circulate for an hour, the blood flowing away from the coronary sinus being restored at intervals to the reservoir. In the course of this hour the blood flow per minute through the coronary cannula gradually increased from 10 to 25 c.c. The whole of the blood in the reservoir was then drawn off and replaced by the blood which had not been circulating. The results are shown in Table III. No change was effected in the rate of the coronary blood, which remained after the replacement of the old by the new blood at 25 c.c per minute. The effect of a large dose of pituitrin is also shown in Table III. When the effect had passed off in one hour it could not be repeated by the further addition of pituitrin.

TABLE III. "Fresh" blood, i.e. blood which has not circulated through the preparation, appears to be without effect on the gradual increase of the coronary flow observed during the course of the experiment.

B.P. = 00. O ₂ saturation constant at 100 %. Pulse 130		"Fresh" blood		$\frac{1}{2}$ c.c. B.D.H. pituitrin to 700 c.c. circulating blood	
Time	Coronary flow	Time	Coronary flow	Time	Coronary flow
1	32	53	43	12	47
10	36	54	44	13	28
20	40	55	46	14	24
30	43	60	47	15	29
45	45	5	47		
50	45	10	46		

Another explanation of this gradual increase which occurred to us was that the initial flow might be below normal on account of the

vaso-constrictor effects of substances in the defibrinated blood used for perfusion. If this were true, however, we ought to have had a considerable drop in the flow in the experiment just quoted, and it would seem that the vaso-tonic effect of fresh defibrinated blood is much smaller and more evanescent, even if present at all, on the coronary vessels than it is on some other vessels of the body, such as those of the lungs and of the kidney. In the latter case the addition of fresh defibrinated blood, as shown by Eichholtz and Verney(11), brings about vaso-constriction. We are unable to give a definite explanation of this gradual rise of blood flow through the coronary vessels. A similar rise is, however, seen on the prolonged perfusion with blood of other isolated organs, and it may be due to a gradual diminution in vitality, or at any rate in functional capacity, of the vascular wall under the abnormal conditions of the experiment. At any rate this gradual increase cannot be looked upon as affording any support for the assumption of the production in the tissues of the heart of metabolites exerting a special dilator action on the coronary blood vessels.

SUMMARY.

1. The coronary vessels are extremely susceptible to change in the oxygen tension of the blood flowing through them, their state of contraction being almost proportional to the oxygen tension of the blood. A fall of the oxygen saturation of the hæmoglobin below 20 p.c. causes maximal dilatation of these vessels.

2. The same maximal dilatation of the coronary vessels is instantaneously brought about by addition of hydrocyanic acid ($M/3000$ to $M/600$) to the blood circulating through them.

3. Both carbonic acid and lactic acid cause a moderate dilatation of the coronary vessels, associated possibly with the alteration in CH of the blood thereby produced.

4. There is no evidence that any other non-volatile metabolites are concerned in the "regulation" of the coronary flow. The increased flow in asphyxia, previously ascribed to the action of metabolites, is sufficiently accounted for by the anoxæmia.

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ON MEASUREMENT OF THE BLOOD-COAGULATION TIME. BY O. S. GIBBS.

(From the Pharmacology Department, University of Edinburgh
and Dalhousie University, N.S.)

IN a previous paper(1) I have described a new form of coagulometer consisting essentially of a broken loop of platinum wire, the coagulation time being that in which a small drop of blood placed on the loop ceases to move when the loop is rotated. A general account of the conditions which cause variation in the observed coagulation time of human blood was also given. In this paper I consider more fully the conditions affecting the coagulation time of the blood of man and of animals both by my own and other methods.

Blood of man.

Contact with the coagulometer. As I have said earlier, the contact with the blood with the instrument should be as small as possible. In Inchley's modification(2) of Buckmaster's instrument(3) and in my own loop method, the contact is reduced to a minimum. With either of these it can be shown that not only the area of contact but the nature of the foreign substance causes great variation in the end-point of coagulation. The following examples in which similar loops of different metals were used to hold the blood, clearly demonstrate this. In each comparison a drop of human blood was freshly drawn by a prick, two loops of different metals were dipped in it simultaneously, and the rate of clotting compared.

	Tungsten secs.	Platinum secs.		Nickel secs.	Platinum secs.
1.	165	109	1.	155	107
2.	154	101	2.	155	102
3.	123	100	3.	145	90
4.	131	94	4.	122	100

Even more striking is the effect of plating a platinum loop with such a metal as copper. The results given below also show the effect of tarnishing, as the end-point gradually becomes quicker, to be delayed again on re-plating.

	Platinum loop no 1 secs	Platinum loop no 2 secs	
1	110	90	
2	95	98	
3	103	91	
4	108	160	Copper plated
5	97	149	
6	96	128	
7	107	156	Re plated

Similar results are to be obtained with the method of Inchley

Further investigation showed that if a wire was used many times it eventually became unreliable, especially with metals such as nickel that tarnish readily. Usually the clotting time tends to shorten. For this reason platinum was chosen for use in my own instrument. An explanation of the difference of end-point on different surfaces is not known, that it is a surface effect is shown (in addition to the above results) by coating a loop with collodion, wax or lacquer, in which case the end-point becomes at once delayed to return to the "normal" (for any given wire) on cleansing

Platinum loops, collodion coated	180 secs
Same loop, cleansed	96 "
Uncoated control	118 "

Glass surfaces probably vary and this would be of importance in such an instrument as Dale and Laidlaw's (4)

All the above results were obtained with my method on human blood, at 37° C (approx.), each pair being done from the same drop of blood. Thorough cleansing and frequent checking of the instrument, especially if unusual results are obtained, is therefore absolutely necessary.

Temperature The work of Dale and Laidlaw shows the importance of the temperature and a study of their curve of temperature effects is most instructive. They conclude "that differences are magnified with the increase of the coagulation time, partly because the end-point loses its sharpness as the process becomes slower." In my own method the slowing of the blood-movement is so pronounced below 30° C that the method will not work, in that of Inchley below 30° C the end-point becomes so sluggish as to be very difficult to determine with certainty. Experiments conducted above 30° C also agree with their results, though I was not able to demonstrate a real difference between 35-40° C even with their own method, and, if it occurs at all, it is only of slight importance. Further Dale and Laidlaw's curve brings out the difficulty of trying to correct for temperature effects, and results so corrected would appear to have little value. One point is certain, viz that, whatever the

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A more exhaustive study was then made for other fallacies in my own method, none however of importance were found. Investigation showed that if two or more samples of blood were taken simultaneously from the same drop they often varied in end point, this occurs with any of the three methods used and no cause can be given, as it does not occur with any particular drop or instrument. The following results obtained with the aid of two other observers emphasise this point.

Method—Inchley (modified, platinum wire) Blood G Order of samples 1, 2, 3
Temperature—37–38° C Time—seconds

	1	2	3
1	265	170	170
2	207	172	227
3	190	245	190
4	222	236	110
5	161	110	221

Dr Inchley kindly supplied me with one of his own instruments which, however, gave the same type of results as above and proved not constant enough for my purpose. A further control was made by using two loops on one stem. This instrument, however, behaves exactly like two separate ones. Dale and Laird's and my own method gives this variation.

Dale and Laird			Gibbs		
1	112	113	1	06	99
2	114	126	2	100	97
3	117	128	3	93	104
4	117	122	4	103	90

Dale and Laird found that pricks with different instruments gave varying results, which they ascribe to the different amounts of tissue juice liberated. I therefore constructed a pricker as in Fig 1, the needles used being "soft tone gramophone", these are very sharp, and a new one can be used for each observation. The depth is adjustable, and thus the pricks are as alike in depth and extent as is possible. Using this instrument, however, one still obtained variations with all the methods.

Blood of animals

It will be seen from what has been said that one can obtain reasonably close results in man and thus one is able to estimate the clotting time of human blood under the arbitrary conditions of the method. Here there is a large area of tissue from which blood can be obtained under practically the same conditions.



Fig 1

by means of a simple prick. In laboratory animals many observers have tried to draw it directly from a vessel, with the idea that the results would be more constant than if it came in contact with the tissues. This may be attempted in various ways, but the results are all along the same lines, though greater variations occur with some methods than with others.

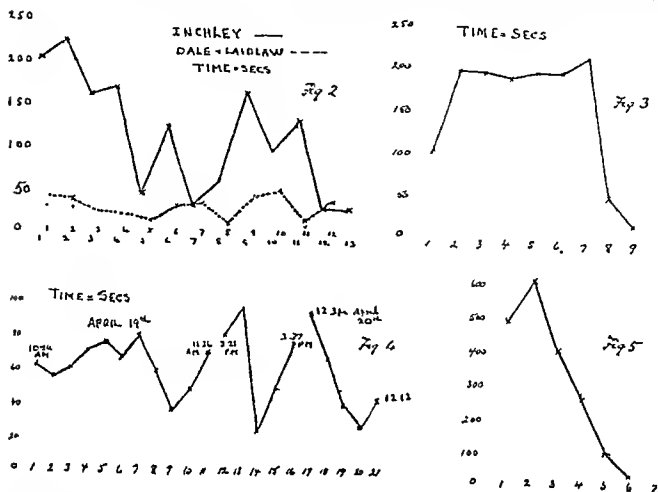
In Fig. 2 the results of a series of estimations of the time of blood-clotting are given; a cat was anæsthetised with paraldehyde, and a paraffined cannula $\frac{1}{4}$ inch in length was tied into the left carotid artery, and a few drops of blood were drawn at intervals for 1 hr. 40 mins. After each bleeding the cannula was cleaned and dried, and the first drops were not examined. The rate of clotting was taken by Inchley's and by Dale and Laidlaw's method alternately. It will be seen that great variations occur with this method, but the general features of the curves are a higher initial point (least contamination) and a more rapid time (greater contamination); this is especially shown by Inchley's method, which commences at 203 secs. and ends at 31 secs. The great variation is due to the different amounts of tissue juice which the blood comes into contact with in its escape. This method then is quite valueless either for measuring clotting under constant conditions, or for forming a blood-curve. The same effects are obtained with a cannula in a vein, though the curve is apt to be more steady when it reaches 30 secs. (less washing out).

Cannon's method(6) of putting a cannula into a branch artery so that the tip just abuts into the main stream leads to the same type of effects, though in this case the fall may not occur for a considerable time if, as he recommends, the cannula is removed after each observation; eventually it occurs as the vessel becomes damaged.

Inchley's method of pricking through the ear vein of a rabbit, allowing the blood to clot in the wound, and re-starting the bleeding by gentle rubbing, or re-pricking, sometimes leads to very constant results for a time. In the end, however, the drop occurs, and is again associated with damage to the vessel easily seen by transmitted light. In Fig. 3 the clotting time is given in a series of observations on a rabbit in which the blood was obtained by Inchley's method from a rabbit's ear, while the rate of clotting was taken by Dale and Laidlaw's method.

Similar results were obtained by my own method and by Inchley's method of estimating the clotting time. Fig. 4 shows the clotting rate of blood obtained from the rabbit's ear by Inchley's method, measured

by my coagulometer; the ear had been carefully cleansed previously with sodium sulphide solution. This experiment was one of the last of



this series and was carried out after experience of this work, with the greatest possible care. It will be seen that the fall in the clotting time occurs if one continues the observations long enough.

Other methods were attempted, such as obtaining the blood from the surface of the kidney, liver or spleen, but the same variation in time resulted, all however tending to a final increase in speed. In these observations the initial time was also low. Blood may be obtained from the under surface of an animal's tongue, or from the comb of a cock, but the same results occur, though in the case of the under surface of a cat's tongue, the initial time is very low.

Cat. Paraldehyde. Blood from pricks in under surface of tongue.

Method—Gibbs. 18 secs., 12, 14, 12, 11, etc.

Blood obtained directly from the heart by means of sharp pointed glass capillary tubes widened out at the top to form a tiny cup 5–8 mm. wide showed the same type of change (Fig. 5). Here a rabbit was anaesthetised with urethane, the heart was exposed under artificial respiration and a series of observations were made on the clotting

time of the blood drawn from the heart at intervals. Inchley's method of measuring the clotting time was used and the temperature was maintained at 37–38° C. In this case one obtained a very typical blood curve, but the method is not suitable for analysis of drug actions or other procedures, owing to the experimental difficulties involved. From these experiments the conclusion was drawn that one could not obtain blood from animals under constant conditions, and that sooner or later the time of clotting would become shorter than in the beginning.

With reference to Inchley's results following the ionic administration of calcium, it will be seen that the speeding up of the clotting time would have occurred in any case whether he gave calcium or not, and that there was not as great a fall as is easily produced in ordinary experiments where great care is taken not to injure the tissues; his results therefore do not show calcium to have any action whatever. These remarks also apply to the paper of Barlow and Ellis(7) whose experiments are open to other serious objections. I feel that their results need to be confirmed by other methods before being accepted.

Determination of the coagulation time may be made with a view of determining variations in the circulating blood, or variations in the power of arresting hæmorrhage. It is to be noted that when blood is obtained direct from a vessel, no certain conclusion can be drawn as to the behaviour of that from a puncture or hæmorrhage since this is mainly dependent on the action of the tissue products in the blood.

SUMMARY.

1. Observations were made on the coagulation time of blood by the methods of Inchley, Dale and Laidlaw and by my loop method. They show that considerable variation of time occurs with the different methods.

2. The time of coagulation of a drop of human blood obtained by pricking varies with the area of contact with foreign material, with the nature of the material, and, as shown by Dale and Laidlaw, with the temperature.

3. When blood is obtained from a blood vessel of an animal either by means of a cannula, or by pricking an ear vein, the coagulation time is inconstant and in the course of an hour or two decreases. These methods give no certain indication of the degree to which hæmorrhage is prevented by hæmostatic remedies in the body when small blood vessels are severed;

re-investigate the question, using more accurately standardised methods than those used by Lalou and others.

Methods. All experiments were performed on dogs anaesthetised with chloraloso (·1 gm. per kg.). Secretin was prepared in the ordinary way, either from the animal's own intestine or from that of other dogs. The injection of secretin was made from a burette connected with the jugular vein. By regulating the inflow of secretin the rate of pancreatic secretion was kept as constant as possible. The juice was collected in test-tubes under paraffin and occasionally samples of blood were taken from the femoral artery; the blood was also collected under paraffin. The following analyses were made:

In the blood. Carbon dioxide content by Van Slyke's method. pH of whole blood by Dale and Evans' method.

In the pancreatic juice. Carbon dioxide content and pH by the same methods. Total alkalinity by means of addition of excess of standard acid, and back titration with standard alkali, using methyl orange as indicator. Amylase, by means of the Achromic Point method. Trypsin, by means of Sörensen's formalin method, after activation by addition of enterokinase. The method employed for determination of lipase needs to be described in detail, since we are not aware that it has been used before.

Since it seems generally agreed that the concentrations of trypsin and amylase show the same rate of diminution in protracted secretion, in only a few experiments were both determined. In all experiments the changes in concentration of lipase were compared with those of amylase.

The methods which are generally used for estimation of lipolytic activity are based on titration with a standard alkali of the fatty acids produced in the digestion mixture. Usually the pancreatic juice, with or without the addition of bile, is added to a watery solution of a lower or to an emulsified fat. In the latter case, the degree of emulsification should be well standardised, but such a procedure is difficult and uncertain. In both cases the digestion mixture quickly reaches a high H-ion concentration, which is detrimental to the enzyme, the optimal H-ion concentration for pancreatic lipase is about 10^{-8} . The obvious source of error was eliminated in our experiments by the use of buffered solutions which allowed only a small change in pH as it proceeded. In a preliminary experiment we were able to show that in digestion mixtures of the same initial pH, one buffered as well as the other unbuffered, the degree of hydrolysis in

OBSERVATIONS ON PANCREATIC SECRETION.

By G. V. ANREP, JOAN L. LUSH AND M. GRACE PALMER.

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THE experiments of Terroine(1,2) and his collaborators, showed that in protracted secretion of the pancreatic juice the concentration of all three enzymes underwent a considerable diminution. This diminution, however, did not affect the three enzymes to the same extent; while the decrease in concentration of the proteoclastic enzyme and of amylase was gradual, the concentration of lipase showed a steep decline and practically disappeared by the end of the experiment. These observations are supported by the experiments made in Pavlov's laboratory by Walther(3), who used animals with permanent fistulæ of the pancreatic duct; he also found that the concentration of the three enzymes does not run parallel, that of lipase always decreasing most rapidly. Walther's experiments, however, cannot be accepted without reservation, since they were made before the discovery of enterokinase and of the reinforcing action of bile upon lipase. Also, in the experiments of Terroine, Lalou and others, although enterokinase was used, the lipase was not reinforced with bile salts.

If Terroine's observations upon the rapid diminution of concentration of lipase are correct, it would seem either that the amount of lipase stored in the pancreas is much less than that of the other two enzymes, or possibly that the cells are less efficient in the production of lipase.

Subsequent experiments performed in Pavlov's(4) laboratory upon animals with permanent or temporary fistulæ under widely different conditions, such as digestion of different food-stuffs in the one case, and administration of acids and stimulation of the vagus in the other, showed complete parallelism in the changes in concentration of all three enzymes; but no long experiments where secretion was protracted by injection of secretin were reported. It might, therefore, be concluded that the difference in the result can be explained on the grounds that secretin has a specific effect on the pancreas causing a quick exhaustion of the lipase without affecting the other two enzymes.

In view of the discrepancy between the results we undertook to

re-investigate the question, using more accurately standardised methods than those used by Lalou and others.

Methods. All experiments were performed on dogs anaesthetised with chloralose (·1 gm. per kg.). Secretin was prepared in the ordinary way, either from the animal's own intestine or from that of other dogs. The injection of secretin was made from a burette connected with the jugular vein. By regulating the inflow of secretin the rate of pancreatic secretion was kept as constant as possible. The juice was collected in test-tubes under paraffin and occasionally samples of blood were taken from the femoral artery; the blood was also collected under paraffin. The following analyses were made:

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Since it seems generally agreed that the concentrations of trypsin and amylase show the same rate of diminution in protracted secretion, in only a few experiments were both determined. In all experiments the changes in concentration of lipase were compared with those of amylase.

The methods which are generally used for estimation of lipolytic activity are based on titration with a standard alkali of the fatty acids produced in the digestion mixture. Usually the pancreatic juice, with or without the addition of bile, is added to a watery solution of a lower ester or to an emulsified fat. In the latter case, the degree of emulsification should be well standardised, but such a procedure is difficult and never certain. In both cases the digestion mixture quickly reaches a very high H-ion concentration, which is detrimental to the enzyme, since the optimal H-ion concentration for pancreatic lipase is about 10^{-8} .

This obvious source of error was eliminated in our experiments by the use of buffered solutions which allowed only a small change in pH as digestion proceeded. In a preliminary experiment we were able to show that in two digestion mixtures of the same initial pH, one buffered as described below and the other unbuffered, the degree of hydrolysis in

the buffered solution was six times as great as that in the unbuffered; in the former case, under the conditions of our experiments, about 25 p.c. of the available ester was hydrolysed when the pH shifted from 7.8 to 7.0.

The method adopted was, shortly, as follows. Glycerol triacetate was used as substrate. A strongly buffered phosphate solution of pH 7.8 or 8.0 was used to stabilise the H-ion concentration. A few drops of a suitable indicator (usually phenol red) were added to the digestion mixture and the time taken to produce equal changes in the pH of the mixture, as shown by the indicator, was used as a measure of the lipoclastic activity. The shift in the pH was traced only as far as pH 7.0, further change being considered too remote from the optimal pH. The changes were best determined by the use of the Dale and Evans' comparator method. In most of the experiments this method was checked by back titration, using phenolphthalein as indicator. Both methods always gave entirely comparable results.

It is obvious that the use of a buffered solution in this method is intended not only to keep the reaction of the medium close to the optimal, but also to show the rate of hydrolysis. The addition of natural bile to the digestion mixture is incompatible with any colorimetric method because of the strong colouration of the medium with bile pigments. We therefore tried other substances in place of the bile. Saponin and caprylic alcohol were tried first, on account of their effect on surface tension, similar to that of bile. They were found to be without any reinforcing effect upon lipase. Synthetic bile salts were used next and sodium glycocholate proved to have as strong an action as bile itself (Exp. 1).

After trying various proportions of glycerol triacetate, buffer solution, bile salts and pancreatic juice, the following mixture was found to give the best results:

- 5 c.c. 1 % glycerol triacetate (freshly prepared).
- 5 c.c. phosphate buffer solution as used in Dale and Evans' method.
- 5 c.c. ·3 % sodium glycocholate.
- 3 c.c. pancreatic juice.
- 8 drops ·02 % phenol red or of another suitable indicator.

Average samples of pancreatic juice gave a change from pH 7.8 to pH 7.0 within about an hour, and we found that under these conditions only about 25 p.c. of the available glycerol triacetate was hydrolysed. Control tubes containing the same mixture without the addition of pancreatic juice showed only a negligible amount of hydrolysis.

Exp 1

Time taken to produce
change from pH 8.0-pH 7.0

Digestion mixture containing		
5 c.c. 1% glycerol triacetate	+ 0	190 mins
5 c.c. phosphato buffer	+ 5 c.c. of 3% saponin	190 "
3 c.c. pancreatic juice	+ 3 drops of caprylic alcohol	185 "
8 drops phenol red	+ 5 c.c. of 3% sodium glycocholate	45 "

It is evident from this experiment that the reinforcing action of bile salts is not due simply to surface tension phenomena causing a greater dispersion of the lipase, but to a specific chemical action of the bile salts.

Exp 2 is an example of the group of experiments in which variations in the rate of hydrolysis obtained by increasing the concentration of the pancreatic juice in the digestion mixture were studied, and a comparison made between the rate in unbuffered and in buffered digestion mixtures

Exp 2	c.c. of pancreatic juice	Time taken to produce a change from pH 8.0 to				
		pH 7.8	pH 7.6	pH 7.4	pH 7.2	pH 7.0
Usual mixture but with water in place of the buffer solution	0.3	Changed to below pH 6.0 in 5 mins				
Usual mixture	0.3	27	60	100	180	300
Do	0.2	38	00	150	250	—
Do	0.1	65	170	290	—	—
Do	0.0	Changed to about 7.9 in 300 mins				

It is obvious from this experiment that in the unbuffered mixture the H ion quickly reaches a value so high that it would hinder further action of the lipase

Changes in enzyme activity of pancreatic juice in protracted secretion

In the seventeen experiments performed it was quite clear that all three enzymes suffered a proportionate diminution in concentration. The following two experiments illustrate the point

<i>Exp 3</i>	Amount of juice secreted	5 c c	10 c c	20 c c	30 c c	40 c c	
	Lipase, time taken to effect a change from pH 8-7.0	45 mins	65 m	50 m	80 m	145 m	
	Amylase, time taken to reach the achromic point	60 secs	90 s	80 s	130 s.	300 s	
<i>Exp 4</i>	Sample	1	2	3	4	5	6
	Amount of juice secreted	25 c c	50 c c	100 c c	125 c c	150 c c	175 c c
	Lipase time taken to effect a change from pH 8.0-7.0	75 mins	80 m	100 m	110 m	250 m	300 m
	Amylase, time taken to reach the achromic point	70 secs	77 s	90 s	122 s	200 s	285 s

In the same experiment the tryptic activity showed a diminution of the following degree. To produce a digestion equivalent to 10 c.c. N/10 NaOH

Sample	1	3	6
Took	7 hrs	9½ hrs	15 hrs.

The change in concentration of the three enzymes does not show a greater rate of diminution in the lipase than in the other two enzymes. These results confirm the results of Pavlov's school, and are in contradiction to the views held by Terroine. There is no need to give further examples since the results of all our experiments were concordant on this point.

It is well known that preparations of secretin made in exactly the same way excite a different amount of secretion in different animals. It has generally been attributed to differences in the store of secretin which the animal's intestine contained before the experiment, or to slight variations in the process of extraction. On several occasions the same preparation of secretin was tested on different animals, and on the other hand several preparations of secretin were tried on the same animal. It was found without any doubt that the most important factor in producing a good secretion lay in the preparation of the secretin; but at the same time, on several occasions we found that in some animals secretion was provoked in large amounts even with small doses of secretin, while in others the same secretin caused only a scanty secretion of juice. In none of our experiments was any relation observed between the amount of juice secreted and the rate of diminution of enzyme concentration. In some experiments the enzymes showed a considerable diminution with a total secretion of only 12 c.c. juice; in others, the same diminution was obtained only after a secretion of over 175 c.c. Thus the rate of diminution of enzyme concentration does not depend on the amount of juice secreted. Moreover, little evidence was obtained to show that the rate of diminution of enzyme concentration depends on the rate of secretion. If, however, two successive small samples secreted at a different rate were analysed, it was found in some experiments that the juice secreted most rapidly was richer in enzymes than that secreted at a slower rate. To show this effect, the experiment has to be performed on a pancreas that secretes well, otherwise it is masked by the progressive diminution in the enzyme concentration. These experiments extend the Heidenhain phenomenon to the pancreatic gland. Exp. 5 illustrates this point.

Exp. 5.				Sample	1	2	3
Amount of juice collected	6 c.c.	6 c.c.	6 c.c.
Time taken for juice to be secreted	90 mins.	40 m.	36 m.
Lipase; time taken to produce a change from pH 7.7-7.0	50 mins.	28 m.	45 m.

The change in the rate of secretion from 90 mins. to 40 mins. was accompanied by an increase in the concentration of the enzymes. The

<i>Exp. 7.</i> Amount of juice secreted in c.c.	Hæmo- globin %	Titratable alkalinity in N/10 H ₂ SO ₄	CO ₂ content of juice in vols. %	CO ₂ content of blood in vols. %	pH of arterial blood	Lipase; change from pH 7·8-7·2
25	100	·153N	322	56	7·5	40 mins.
50	105	·149	324	54	7·46	—
75	97	·144	322	55	7·44	—
100	100	·144	281	44	7·34	—
125	103	·141	255	41	7·37	50 "
Intravenous injection of 60 c.c. of 10 % sodium carbonate						
150	87	·147	335	95	7·74	135 "
175	100	·141	305	69	7·77	210 "
Rest for 45 mins.; injection of 60 c.c. of 10 % sodium carbonate						
200	89	·145	397	124	7·78	85 "
225	84	·143	307	74	7·77	80 "

45 mins. was allowed, the concentration of lipase increased sharply. Since, during the period of rest, 60 c.c. of Na₂CO₃ were injected slowly into the blood, we had to make sure that the latter was not the cause of the rise in concentration of the enzymes.

In several experiments resting periods were interposed with periods of injection of secretin. It was found that after every resting period the juice was much more concentrated. Since it is known that the vagus nerve has a potent influence upon the excretion of enzymes, we tried to find whether this augmentation in enzyme concentration after rest is in any way dependent on the integrity of the vagus nerve. The experiments showed that the enzyme concentration in the juice increased after rest, even when the vagi were cut after administration of atropine or both. Stimulation of the vagus with Faradic current did not, in our hands, cause any larger increase in the enzyme concentration than was caused by rest alone. In this respect our results differed from those of Savich(5), who showed a definite increase in concentration of the enzymes during the period of stimulation of the vagus. His experiments

<i>Exp. 8.</i> Amount of juice in c.c.	Lipase; time taken to produce a change from pH 7·8-7·0	Titratable alkalinity of juice
2	20 mins.	·141N
4	27 "	·134
6	33 "	·132
Vagi cut; 15 mins. rest		
8	18 "	·122
10	40 "	·124
12	190 "	·106
Rest for 30 mins.		
14	43 "	·108
16	58 "	·111
18	350 "	·105
Vagus stimulated for 30 mins.		
20	50 "	·111
22	70 "	·111
24	350 "	·120

were conducted on spinal animals. Our experiments also differ from those of Babkin(7), who obtained the effect of the vagus on animals anaesthetised in a similar way to ours but with both splanchnic nerves cut. However, it is evident that the augmentation in the concentration of the enzymes, which was observed in our case, was independent of the vagus mechanism.

That the vagus nerve does not regulate the re-establishment of a gland during the periods of rest is shown also by the experiments in which both vagi were cut during the period of secretion. If this is done at the very beginning of secretion, without discarding the first few cubic centimetres of juice, it causes a considerable diminution in the concentration of enzymes, which proves once more an old observation of Pavlov. If, however, the vagi are cut after secretion has proceeded for some time, it has no effect on the concentration of enzymes. This observation supports the experiments of Savich and others.

On looking through our experiments we find that the only possible explanation of the facts described in this communication is found in the difference of the general state of the experimental animal. Those animals which showed only a gradual and incomplete recovery of blood pressure after injection of secretin, secreted badly and the enzyme concentration was low. Animals which kept their blood pressure high to the end of the experiment were of the type which secreted much juice and in which the enzyme concentration fell slowly. Along with Babkin's experiments this shows once more the importance of a good blood supply to the pancreatic gland. During the periods of rest the blood pressure improved considerably, and this seems to be the only explanation of the apparent recovery of the gland.

The above explanation is corroborated by the observation that after injections of histamine, secretin causes a much smaller secretion of pancreatic juice and the juice contains considerably less enzymes (Exp. 9).

Exp. 9. Dog 8 kilos. Four injections of 1 c.c. of secretin each caused a secretion of 1.2, 1.5, 1.3, and 1.4 c.c. of juice. Between each injection the blood pressure was allowed to recover to normal (120 mm. Hg).

After an injection of 0.2 mgm. of ergamine diphosphate the blood pressure fell to 40 mm. and did not recover for a long time. Injections of the same dose of secretin caused a flow of juice of only 0.2, 0.35, 0.1 and 0.12 c.c. After an interval of one hour the blood pressure had risen to 80 mm. and the secretion improved.

The concentration of enzymes was very considerably less in the juice which was collected during the period of low blood pressure. When the blood pressure and the secretion recovered the concentration of the enzymes rose nearly to the previous level.

CONCLUSIONS.

1. In protracted secretion of pancreatic juice maintained at a constant rate by continuous injection of secretin, all three enzymes show a parallel diminution in their concentration.

2. Contrary to the view of Terroine and Lalou, the lipase does not show a more rapid fall in concentration than the other two enzymes.

3. This diminution in concentration of enzymes is not a true exhaustion of the gland as is shown by experiments with pilocarpine.

4. The lipase was estimated in these experiments by a new standardised method which should prove of value in experiments dealing with any lipoclastic activity.

5. The progressive fall in the concentration of enzymes does not depend upon the alkalinity of the juice, the alkalinity of the blood, or on the absolute amount of juice secreted; neither can it be explained by changes in the rate of secretion.

6. After a short rest the pancreatic gland secretes a juice with a higher concentration of enzymes. The effect of rest is independent of the vagus mechanism, and occurs also after administration of atropine and after section of both vagi.

7. The explanation is advanced that the changes in the concentration of the enzymes depend upon the general condition of the animal and the blood supply to the gland.

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THE PART PLAYED BY THE ALA CINEREA
IN VASO-MOTOR REFLEXES.

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Hospital and Medical College.)

ROBERTS and I⁽¹⁾ have confirmed the results obtained by Ranson and Billingsley⁽²⁾ that a vaso-depressor effect is obtained on electrical stimulation of a point close to the calamus scriptorius (the depressor point) and we have discussed the question whether it represents merely a point on the depressor arc or is the supreme vaso-dilator centre. The present paper offers additional evidence as to the nature of this point. I owe the suggestion that I should try the effect of application of strychnine to the floor of the fourth ventricle to Prof. Langley, to whom my thanks for this and other help are due.

Application of 1 p.c. strychnine to the depressor points.

In 1888 Roy and Aducco⁽³⁾ showed that strychnine paralysed the depressor in the rabbit and the vagus in the cat and dog. Bayliss⁽⁴⁾ in 1908 described reversal after injection of strychnine of the normal effect obtained from the depressor in the rabbit and the vagus in the cat. Langley⁽⁵⁾ contested this in 1912, saying that the effect of strychnine is, after a preliminary stage of increase of excitability, to decrease reflex vascular effects whether pressor or depressor.

Experiments were performed on cats, the anæsthetics used being either C.E. mixture or urethane. The results obtained do not depend on the anæsthetic. Two experiments were also performed on rabbits, urethane being used as the anæsthetic. The procedure was that, a tracheal cannula having been inserted, the right carotid artery and depressor nerve (or, if it could not be separated, the vagus) and then the right sciatic nerve or the brachial nerves were isolated. The floor of the fourth ventricle was exposed by the operation already described⁽¹⁾. A blood-pressure record was taken from the carotid and the optimum strengths of faradic stimulation (2 volts in primary) for a good effect from the depressor and good pressor and depressor effects from the sciatic were noted. Strychnine nitrate in 1 p.c. solution in distilled water warmed to body temperature

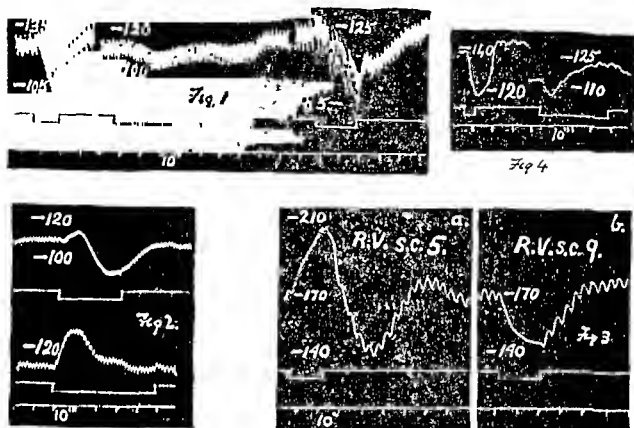
was now applied on a minute pledget of wool to the posterior part of the floor of the fourth ventricle; the application was confined as nearly as possible to the posterior part of the *alæ cinereæ* (depressor points); the amount of fluid used would contain about $\cdot 05$ mgm. of the salt. During this application graphic records were obtained at intervals of the results of stimulation of the above nerves with the same strengths of stimuli as had previously been used.

Stimulation of the depressor nerve and of the vagus. On applying the strychnine there is a transient period in which the depressor effect may be increased, followed sometimes by an interval in which it can be observed to diminish progressively; it then practically disappears (Fig. 1). Reflex cardiac and respiratory effects from the vagus are blocked at the same time. Then vagal reflexes slowly reappear provided the application has been of short duration. The reappearance may be marked by a stage in which the depressor effect is exaggerated. A reversal of effect, such as described by Bayliss⁽⁴⁾ with the depressor in the rabbit, was not obtained. The nearest approximation to his result is shown in Fig. 2. This was obtained once only. As will be seen, the slight primary pressor effect was much increased by strychnine and the depressor effect was abolished. In this experiment, after recovery from strychnine, I split the vagus into two bundles (there was no separate depressor nerve). One bundle caused mainly a pressor effect, the other mainly a depressor effect. The nerve therefore contained pressor as well as depressor fibres. Most observers who have stimulated the vagus in the cat have found that occasionally it causes a rise instead of a fall of blood-pressure. As a demonstration that both pressor and depressor fibres may be contained in the vagus of the cat a fortunate tracing (Fig. 3), showing the result of stimulation of the vagus in one animal, is appended. In this isolated case when the secondary coil was at 5 a pressor effect followed by an after-fall was obtained; while, when the secondary coil was at 9, a pure fall was given.

An apparent "reversal" of effect of the depressor after application of strychnine to the *alæ cinereæ*, was obtained in the rabbits (Fig. 4), but in both cases there was some evidence, as indicated by a slight initial rise or an after-rise, that the depressor contained some pressor fibres. It may be noted that Stewart and Pike⁽⁶⁾ found the first reflex effect obtainable from the depressor of the rabbit (and vagus in the cat) in resuscitation from cerebral anæmia to be a rise of blood-pressure.

The results, I think, corroborate Langley's explanation⁽⁵⁾ that the substitution of the depressor by a pressor effect (Bayliss' result), when

it is obtained, depends upon the presence of pressor elements in the nerve stimulated.



Figs. 1-6. Effects of application of strychnine to the lower part of the floor of the fourth ventricle (approximately to the depressor points).

Fig. 1. Stimulation of left vagus of cat (right vagus intact) before, during, and in recovery from, strychnine.

Fig. 2. Stimulation of left vagus of cat (right vagus cut) before and after strychnine. Increase of pressor, abolition of depressor effect ("Reversal").

Fig. 3. Stimulation of right vagus of cat (left vagus intact). Pressor and depressor effect from the same nerve: (a) sec. coil at 5, (b) sec. coil at 9 cm.

Fig. 4. Partial reversal of depressor reflex in the rabbit. Stimulation of left depressor nerve before and after strychnine.

Stimulation of sciatic nerve. Some difficulty was experienced in getting from the central end of the cut sciatic nerve a depressor reaction of such magnitude and constancy as to be perfectly reliable. Unless one could produce this reaction with certainty one would not be justified in attributing its absence on any occasion to a specific agency. Various methods were tried. Weak faradic currents as well as partial blocking of the stimulus were used, as described by Reid Hunt(7); I was not satisfied that the depressor reflex obtained by this means was constant enough for my purpose. Bilateral section of the pressor path in the region of the posterior horns of the cord was tried, as described by Ranson and

Billingsley(8); this had to be combined with exposure of the floor of the fourth ventricle in one acute operation, which was found to be too severe for the animal. Gruber(9), Gruber and Kretschner(10), and Vincent and Ogata(11), laid stress on the use of very low rates of faradic stimulation for the production of a depressor response. I have been able to produce this effect with certainty by the same means, provided that a few minutes (2-5) were allowed to elapse before repeating the excitation, care also being taken to avoid any asphyxia(12). Under these circumstances one could feel confident that if the depressor response disappeared during the course of the experiment, its disappearance was due to something occurring at that time. Fig. 5 shows a graphic record of the normal depressor response obtained from the sciatic under such conditions; the primary current was interrupted by a rod vibrating twice a second and the secondary coil was at 5 cm. 1 p.c. strychnine nitrate in distilled water warmed to body temperature was then applied on a minute pledget of wool to the depressor points, and tracings were taken at intervals with the same frequency and strength of stimulus. The depressor response soon disappeared (cp. Fig. 5); the blocking was commonly preceded by

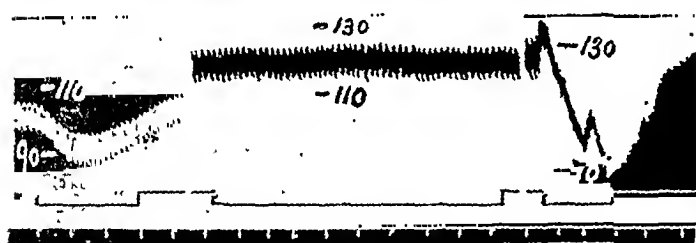


Fig. 5.

Fig. 5. Stimulation of sciatic of cat, as in text, sec. coil at 9 (one vagus intact), before application of strychnine, during application and during recovery.



Fig. 6.

Fig. 6. Pressor reflex from sciatic, tetanising current, 12 secs., sec. coil at 4, during abolition of depressor reflex by strychnine.

first an exaggeration and then a rapid lessening of the response. Respiratory reflexes from the sciatic were abolished at the same time. After removal of the strychnine pledget the depressor and respiratory reflexes reappear at about the same time as does the depressor reflex from the vagus. The reappearance of the sciatic depressor response is accompanied by a stage in which it is exaggerated (as shown in the figure), after which it returns to normal.

During the absence of depressor effect noted above, pressor reflexes

can still be obtained from the sciatic (Fig. 6). The effect of strychnine in these experiments is not due to a general action on the cord after absorption into the blood stream, for depressor responses can be abolished without the occurrence of convulsions, and injection of the total amount in the pledget does not abolish depressor responses. The result, I think, shows that in the normal animal the depressor impulses from the sciatic pass through the lower part of the spinal bulb and do not, or only to a trifling extent, depend on a spinal reflex. It is possible, however, that the simultaneous abolition by strychnine of the bulbar respiratory reflexes may aid the abolition of the sciatic depressor response, in the causation of which the rapid shallow respiration occurring on sciatic stimulation has sometimes been said to play a part (13).

Stimulation of the depressor points. It might be supposed that the action of strychnine on the depressor point is due to an effect on the superficial nerve cells of the region, *i.e.* to cells forming part of the dorsal nucleus of the vagus. But paralysis of the nerve cells (or of their synapses) would not prevent their axons from being stimulated at the point where they leave the cell. In fact, however, strychnine also diminishes the effect of direct stimulation of the depressor point (Fig. 7). Its effect on the cells must therefore be associated with either abolition of excitability of the superficial nerve fibres or an action on neighbouring or more deeply lying nerve cells.

Provided the strychnine is not allowed to act on the medulla for too long the depressor points recover their excitability to direct stimulation (Fig. 8); this recovery is coincident with the return of the depressor reflex from the vagus.

The question discussed by Ranson and Billingsley (8), by Bayliss, and by Roberts and myself, whether the depressor point is a supreme vaso-dilator centre, does not in any case arise; observations detailed in the next section show that the depressor points may be destroyed by cauterisation without diminishing the depressor effect from the sciatic, so that the depressor point cannot be a supreme vaso-dilator centre, for

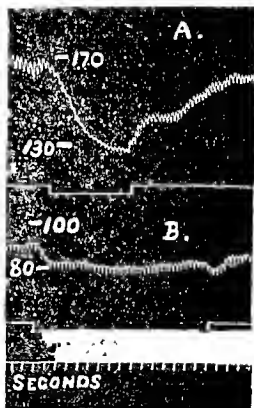


Fig. 7. Stimulation of left depressor point, cat, both vagi intact; (A) normal, (B) after strychnine.

not all dilator reflexes are transmitted through it. It may be that the abolition of sciatic depressor effect is due to diffusion to the vaso-constrictor (or pressor) centre, causing predominance of pressor action by

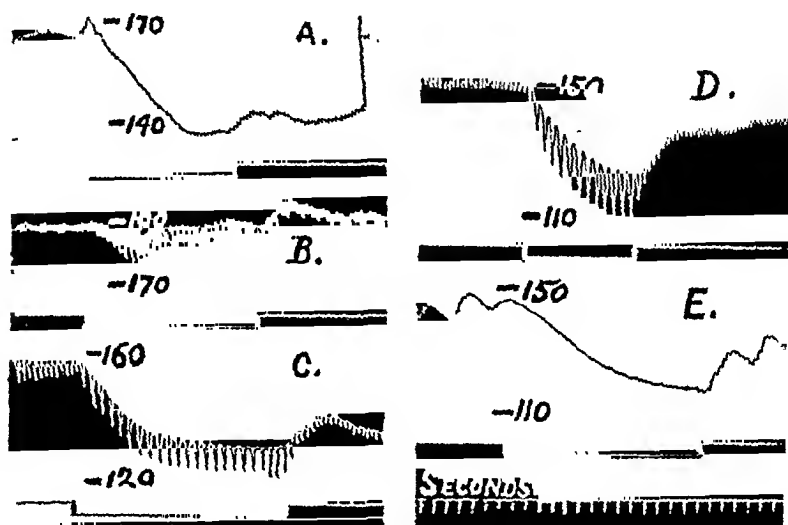


Fig. 8. Increased cardio-inhibition occurring during recovery from strychnine; stimulation of right depressor point; vagi intact; s.c. 12 throughout. A. Normal: strychnine was then applied for 10 mins. B. Stim. 5 mins. later. C. 20 mins. after B. D. 50 mins. later. E. Stim. after section of vagi.

increasing its excitability. But I believe that strychnine acts on the cells and possibly the fibres of the depressor point and that it diffuses to, and acts on, a nucleus or part of a nucleus¹ through which depressor reflexes from the sciatic are transmitted—a nucleus which is in no wise affected by cauterisation of the depressor points; but till the true nature of the depressor reflex from the sciatic is established no definite conclusions can be reached.

Cauterisation of the pressor and depressor points.

In this series of experiments, which deals with cats anaesthetised with urethane, the floor of the fourth ventricle was exposed in the way already described, and blood-pressure tracings were taken of the depressor effect from the vagus or depressor nerve and of the pressor and depressor effects from the brachial nerves at the elbow or the sciatic nerve.

The depressor points were then identified by electrical stimulation;

¹ In this connection one thinks naturally of the posterior extension of the dorsal accessory-vagus nucleus into the "closed" medulla.

they were cauterised by applying the end of a hot copper wire three times to them and the nerve stimulation was repeated with the same strengths of current as before. The pressor points were then identified, and cauterised in the same way; artificial respiration was started, for the latter procedure interfered with normal respiration, and the nerve stimulation was repeated. A complete series of tracings is shown in Fig. 9.

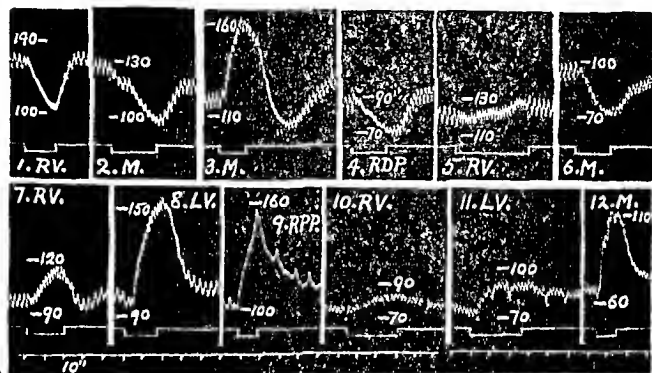


Fig. 9. Effect of cauterisation, cat. 1. Stim. R. vagus (L. vagus intact, s.c. 8). 2. Stim. R. median (s.c. 9; 2 per sec.). 3. Stim. R. median (s.c. 4, magnetic interruptor). 4. Stim. R. depressor point (s.c. 10).

Depressor points cauterised.

5. Stim. R. vagus (s.c. 8). 6. Stim. R. median nerve as in 2. 7. Stim. R. vagus as in 3. 8. The L. vagus was cut and stim. as in 3. 9. Stim. R. pressor point (s.c. 10).

Pressor points cauterised.

- Artificial respiration 10 and 11. Stim. R. and L. vagi as in 7 and 8. 12. Stim. median as in 3.

Cauterisation of the depressor points caused a fall followed by a rise of blood-pressure. After cauterisation, or indeed any injury, of the depressor points, direct stimulation of the injured area did not evoke any depressor effect.

From the depressor nerve after cauterisation of the depressor points either no effect or, if the strength of the stimulus was increased, a pressor reflex was obtained instead of the usual depressor one. The "reversal" was obtained every time. The difference between this result and the partial reversal, occasionally observed after application of strychnine

to the depressor point, may be explained by the hypothesis that in the case of strychnine, diffusion to, and paralysis of, the connections of some, at least, of the pressor as well as the depressor fibres of the vagus may occur. The phenomenon of reversal after cauterisation of the depressor points can only be due to destruction of depressor and unmasking of pressor fibres. The close resemblance between the effect occasionally produced by strychnine and that of cauterisation is suggestive evidence that in the former as in the latter case, "reversal," when it occurs, is due to the unmasking of pressor fibres. The diffusion hypothesis mentioned earlier will account for another and more striking difference between the effects of cauterisation and those of application of strychnine. After cauterisation appropriate stimulation of the median nerves or sciatic still causes a fall of blood-pressure, an effect which is abolished by local application of strychnine.

The facts recorded in the preceding paragraph clearly indicate that the depressor points are points on the reflex depressor arc of the vagus and are not essential for the production of vaso-dilatation. In the course of his experiments on animals decerebrated by the injection of starch Langley⁽⁵⁾ has made observations which he interprets as possibly indicating that the centres for reflex depressor effects from the vagus are distinct from those involved in depressor effects from the sciatic. This dissociation may be due to the blocking by starch of an arteriole serving one capillary district within the area of a "centre," while another district close by is not so blocked off. It might well be produced if the depressor point or a cell station on the vagal depressor arc were blocked off without interfering with the nutrition of the neighbouring point which is not affected by cauterisation of the depressor points, but which is affected by the diffusion of strychnine applied thereto; or *vice versa*.

It has been shown earlier⁽¹⁾ that the pressor points are not constant in position. Their situation was therefore determined in each case by experiment. Cauterisation of the pressor points caused a rise of blood-pressure followed by a fall. After cauterisation of the depressor points, pressor reflexes only, as has been said, can be obtained from the central end of the cut vagus. If now the pressor points are cauterised these vagal pressor effects are reduced to about one-third. At the same time the pressor effect from the median is almost unchanged, this in spite of the fact that after cauterisation of the pressor points, which are close to the fasciculus solitarius, there is considerable interference with respiration and artificial respiration may have to be employed. Here we have similar direct proof that the pressor points do not represent the bulbar vaso-

three other experiments, after cauterisation of the depressor points, the "reversal" of the depressor reflex from the vagus into a pressor reflex was demonstrated, and then the minimal strength of stimulus which would just give a pressor reflex from the vagus was determined. A square millimetre of filter paper soaked in warm 0.1 p.e. strychnine nitrate, was then applied to the upper end of each *ala cinerea*, where the pressor point is normally found, and in each case, after the lapse of about 5 minutes, a definite increase in the size of the pressor reflex from the vagus was noted, this increase lasting for about the same period after removal of the strychnine plug (Figs. 11, 12). Distilled water had no such effect. The effect of Ringer was not tried. It may therefore be concluded that the action of small doses of strychnine nitrate of the order of about .005 mgm. locally applied to the appropriate sites is to increase vascular reflexes whether pressor or depressor.

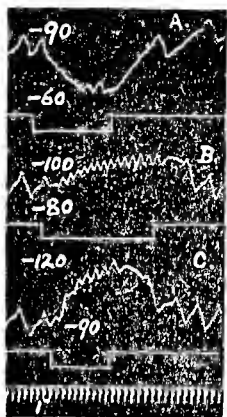


Fig. 12. Cat, both vagi cut; stim. of L. vagus; s.c. 8 throughout. A. Normal. B. After cauterisation of depressor points. C. After application of .1 p.o. strychnine to pressor points.

CONCLUSIONS.

1. The application of 1 p.o. strychnine nitrate in doses of about .05 mgm. in distilled water to the depressor points of the cat's spinal bulb blocks the depressor reflex on vagal stimulation and prevents direct stimulation of the depressor point from having any effect.

2. A fall of blood-pressure can be obtained in the cat by stimulating the sciatic or median nerve with induction shocks repeated two to four times a second. This fall of blood-pressure and the reflex effect of the sciatic on respiration are prevented by the local application of strychnine, as described above.

3. The amount of strychnine applied is too small to paralyse structures elsewhere than in the approximate region to which it is applied, so that normally vagus reflexes and depressor somatic reflexes depend upon some structure or structures in the lower portion of the bulb.

4. The above application of strychnine does not abolish the pressor effect of somatic nerve stimulation.

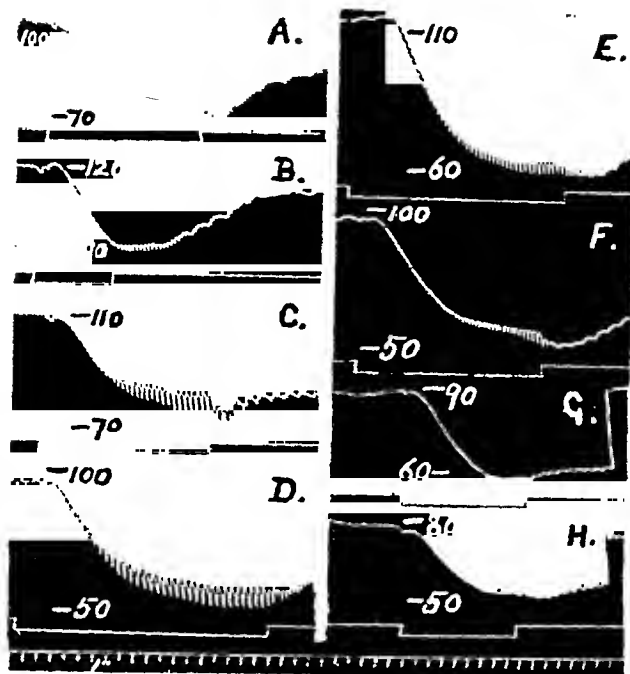


Fig. 10. Stim. of L. vagus (R. vagus intact, s.c. 8). A. Before applying .1 p.c. strychnine to depressor points; B. to E. at intervals during application (about 1 hour). F. 10 mins. after removing strychnine. G. 30 mins. H. 80 mins.

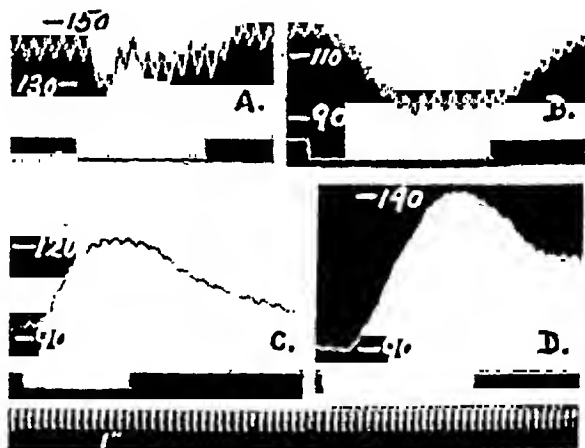


Fig. 11. A. Minimal depressor reflex obtained from L. depressor of cat; L. vagus cut, R. vagus intact; s.c. 29-5. B. Increased reflex obtained after application of .1 p.c. strychnine to depressor points with same strength of stimulus. Half an hour after removal of strychnine the minimal stimulus which gave a depressor reflex was 38. After an interval of 2 hours the depressor points were cauterised. Vagal pressor response: C. before strychnine; D. after strychnine.

THE ACTION OF OXALATES AND OF CITRATES ON
CIRCULATING BLOOD. By J W PICKERING
AND J A HEWITT

(From the Department of Physiology, King's College, London)

MODERN investigation and interpretation of blood coagulation are due to two outstanding observations (1) the preparation of fibrinogen from blood, and (2) the demonstration of the anti coagulant action of soluble oxalates, citrates and fluorides on shed plasma, together with the restoration of coagulability on replacement of the removed calcium. As a consequence it may be said that, apart from the work of Nolf, of Mills, and of the present writers, recent conclusions on the clotting of blood have been based on reactions *in vitro*, without ascertaining whether similar phenomena occur in the living animal. The experiments of Howell(1), however, indicate that it is not yet possible to prepare solutions of fibrinogen of uniform properties, while Pickering and de Souza(2) point out differences between the coagulable material of circulating blood and the fibrinogens of the laboratory.

Experiments will be described showing the influence of both oxalates and citrates on the coagulability of circulating blood and the effects produced by the subsequent intravascular injection of calcium chloride.

Summary of earlier experiments in vivo Previous attempts to decalcify blood by the intravascular injection of soluble oxalates have yielded inconclusive results, owing to the toxicity of oxalic acid and its neutral salts. For example, Mills(3) stated that the blood of a rabbit completely prostrated by the intravenous injection of potassium oxalate, exhibited a normal coagulation time. Sabbatani(4), however, demonstrated suppression of clotting in the blood of frogs, dogs and rabbits, after the intravascular injection of sodium citrate and showed that the introduction of a suitable mixture of sodium citrate and calcium chloride into the circulation does not produce this effect. Weil(5) observed hypercoagulability of blood shed after the intravenous injection of small amounts of citrates, an observation which may be interpreted as indicating disturbances of the colloidal complexes of the plasma. This

5. An increase in the above-mentioned reflexes occurs in the early stage of the action of 1 p.c. strychnine, in recovery from the paralysis produced by it, and when 0.1 p.c. strychnine is applied instead of 1 p.c.

6. Destruction of the depressor points by cauterisation has the same effects as applying 1 p.c. strychnine to them, *except* that (a) the depressor effect of somatic nerve stimulation is not altered, from which it is concluded that 1 p.c. strychnine diffuses to some structure in the course of somatic depressor reflexes and paralyses it; and (b) that strong stimulation of the vagus may have a pressor effect, though no depressor one can be produced.

7. The occurrence of a rise of blood-pressure on vagus stimulation after destruction of the depressor point is definite evidence that pressor fibres are present in the vagus. This fact, and the effect on the vascular reflexes obtained by local application of strychnine to the depressor points, confirms the view put forward by Langley that strychnine does not cause any reversal of nerve impulses, *i.e.* does not convert inhibitory into vaso-constrictor impulses.

8. Cauterisation of the pressor area following cauterisation of the depressor points, reduces the pressor action of the vagus but leaves uninfluenced the pressor action of somatic nerves.

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author found also that no less than 1 p.c. of citrate was necessary to maintain the fluidity of blood perfused through the liver, whereas *in vitro* one-quarter of this amount suffices. Working with rabbits, Mills(3) noted that oxalates were more toxic than citrates; in one experiment where sodium citrate was injected, followed by a lethal amount of lung extract, most of the blood remained unclotted for about 24 hours at the temperature of the ice-chest.

The methods and terms employed. Early in the progress of this work it was found impossible to produce incoagulable blood by the injection of soluble oxalates in anæsthetised cats and rabbits, as death occurred prior to the suppression of coagulability. Pithed cats, however, were found to be much more resistant to both oxalates and citrates, and were accordingly employed. The animals were anæsthetised with A.C.E., a trachea cannula inserted, artificial respiration maintained and the brain destroyed. In all cases the ureters were ligatured. When rabbits were used full ether anæsthesia was continued until the death of the animal.

The potassium oxalate and sodium citrate were each dissolved in 0.86 p.c. sodium chloride; the calcium chloride was in aqueous solution. Injections were made either into the jugular vein or, in certain cases, into the ventricles. Blood was shed from the carotid through a paraffined cannula and was collected in wide-mouthed glass vessels, for reasons detailed in another communication (6). The terms "commencement" and "completion" of clotting imply respectively the first visible departure from fluidity other than the appearance of minute filaments on the surface of the blood, and that coagulation has so far advanced that the containing vessel can be inverted without spilling the contents. In the table, the upper figures record "commencement" of clotting, the lower the "completion" of that process. Results are only given when it was possible to inject sufficient oxalate or citrate to induce prolonged restraint of clotting. In a number of other cases death occurred before suppression of coagulability.

Action of oxalates. The intravascular injection of soluble oxalates into the cat may have one of two effects depending on the amount administered. Moderate amounts (0.005 gm. per 3 kilo. animal) cause hypercoagulability; large doses (0.25 gm.) suppress coagulation. The latter effect is similar to that found when blood is decalcified *in vitro*. Furthermore, addition of calcium chloride (*i.e.* recalcification) to such incoagulable blood *in vivo*, produces extensive thrombosis. In this respect also the reaction *in vivo* resembles that *in vitro*. A single experiment is given by way of illustration.

Exp. Tabby cat, male. Weight 3 kilos. Normal clotting times of blood 15 minutes after pithing; commencement 0 mins. 5 secs., completion 7 mins. 5 secs.

The effects of serial injections of potassium oxalate intravenously, followed by intra-cardiac injections of calcium chloride are shown.

Time		Notes	Coagulation times of shed blood	
hrs.	mins.		mins. secs.	
2	35	10 c.c. 0.5 % oxalate injected		
2	39		5	30
			7	10
2	44		4	40
			5	50
2	53		4	40
			5	50
2	53	10 c.c. 0.5 % oxalate injected		
2	58		2	30
			4	20
3	4		2	10
			3	20
3	0	10 c.c. 0.5 % oxalate injected		
3	10		2	10
			3	10
3	21		2	10
			4	20
3	24	10 c.c. 2.5 % oxalate injected		
3	24	Heart stopped		
3	25	Heart resumed beating feebly		
3	31	Arterial blood very venous		
			Fluid after 14 hrs. Found clotted 20 hrs. after shedding	
3	37	2 c.c. 2 % CaCl_2 injected into right ventricle		
3	37	2 c.c. 2 % CaCl_2 injected into left ventricle		
3	39	Blood could not be obtained from carotid		
3	49	Post-mortem examination showed a large clot in right ventricle, small clot in left ventricle. Massive clots were found in the dorsal aorta, inferior vena cava and portal vein		

The intravascular injection of soluble oxalates must be assumed to cause the formation of insoluble calcium oxalate and, according to Nolf(7), the presence of solid, inert material in the blood, such as calcium oxalate, invokes clotting by the formation of so-called thromboplastic centres of coagulation. On this view, the thrombosis resulting from the recalcification *in vivo*, after intravenous injection of potassium oxalate would be due to the formation of thromboplastic centres by the insoluble calcium oxalate present.

The following experiment shows that this conclusion is incorrect. 15 c.c. of a 4 p.c. suspension of crystals of calcium oxalate (of average diameter 4.5μ) in Tyrode's solution, was injected into the circulation of an anaesthetised cat. The animal died, but no intravascular clotting occurred. The crystals were identified in the serum obtained from the shed blood of the animal. They did not, however, *in vivo*, form foci from which coagulation started; on the contrary, the clotting time of blood

shed immediately before the death of the animal was increased by 6 minutes. This suppression of coagulability appears concordant with the observation of Bordet and Delange(s), who found that tricalcium orthophosphate inhibits the coagulation of shed oxalated plasma, and with those of Dale and Walpole(9), who attributed the anti-coagulant action of barium sulphate on oxalated plasma to adsorption of prothrombin.

One of the outstanding features of the observations now recorded is the fact that removal of ionised calcium from circulating blood and subsequent replacement, alters it in such a manner as to produce a change from sol to gel. A strictly analogous change occurs *in vitro*. It seems reasonable to conclude that plasma, *in vivo* or *in vitro*, which has been decalcified and recalcified, is different from a plasma not so treated and in which the colloids associated with calcium have not been disturbed. It need only be indicated that in the former coagulation rapidly takes place, while the latter remains indefinitely fluid.

Results obtained from experiments carried out with oxalated and recalcified plasma cannot, therefore, be strictly applied to the problems of circulating blood or to those of intravascular coagulation.

Action of citrates. Like soluble oxalates, citrates on intravascular injection cause disturbances of the plasma. Moderate amounts of sodium citrate (0.1 gm.) produce hypercoagulability, while introduction of large amounts (1.3 gm.) into the circulation inhibits clotting. In this respect soluble citrates behave similarly *in vitro* and *in vivo* with both the cat and the rabbit. When, however, the effect of recalcifying such citrated circulating plasmas is examined, the cat is found to react differently to the rabbit and it is convenient to cite these separately.

The blood of a cat was rendered incoagulable by the injection of sodium citrate, and during this condition sufficient calcium chloride to neutralise the citrate was introduced into the circulation. No thrombosis resulted. In this animal, therefore, the action of calcium chloride on citrated blood is not the same as on oxalated plasma.

In the rabbit, under otherwise similar conditions, sodium citrate delays coagulation, but subsequent injection of similar amounts of calcium chloride causes intravascular coagulation; an effect resembling that found to occur with oxalates.

In the action of sodium citrate on injection in the cat one noticeable feature is the rapid partial recovery from the toxic effect. Concomitant with this recovery there is a marked increase in the coagulability of the blood when shed on to glass. An explanation of this phenomenon is to

be found in the observations of Battelli and Stern(10) and of Salant and Wise(11), who demonstrated that citrates are destroyed by isolated tissues *in vitro* and are rapidly removed, by oxidation, from the bloodstream after intravenous injection

Assuming that the hypothesis of Sabbatani is correct, viz that the blood calcium is "immobilised" by the addition of sufficient citrate, it would appear that the elimination of citrate is accompanied, or followed, by the re establishment of the calcium in its original combination in the blood. This restoration of coagulability *in vivo* does not give rise to intravascular coagulation

The conclusion seems unavoidable that "recalcified" citrated blood of the cat behaves *in vivo* quite differently to "recalcified" citrated plasma when the calcium chloride is added *in vitro*

SUMMARY.

1 Blood oxalated and recalcified *in vivo* behaves similarly to blood so treated *in vitro*

2 Thrombosis arising from the intravascular injection of calcium chloride into the circulating oxalated blood of pithed cats is not due to the formation of crystals of calcium oxalate in the plasma

3 Intravascular injection of small amounts of sodium citrate into the cat produces hypercoagulability, large amounts produce temporary inhibition of clotting and blood shed during the inhibitory phase remains fluid for several hours

4 Recalcification of citrated blood *in vivo* does not cause thrombosis in the cat but may induce intravascular clotting in the rabbit

5 Deductions from the recalcification of plasmas oxalated or citrated *in vitro* have only a limited meaning and cannot be applied to the problems either of circulating blood or of thrombosis

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THE EXCITABILITY OF THE ANÆMIC MEDULLA OBLONGATA. BY FR. ROBERTS.

(From the Physiological Laboratory, Cambridge.)

Two papers have recently been published by Huggett and Mellanby on the subject of adrenalin apnoea. In the first⁽¹⁾ they joined me in supporting the view that the suppression of the activity of the respiratory centre is due to vaso-constriction. But in the second⁽²⁾ they repudiate this view and now hold that the apnoea is due to a direct paralytic effect of adrenalin on the centre.

Huggett and Mellanby investigated the effect of adrenalin upon certain reflexes obtainable from the bulb (vaso-motor, cardio-inhibitory, swallowing, conjunctival, oculo-motor) and upon certain reflexes obtainable from the spinal cord. They found that none of these were reduced by adrenalin. They argued that if adrenalin caused vaso-constriction the activity of one or more of the centres governing these reflexes would have been diminished. Since they were not diminished, they concluded that adrenalin did not cause vaso-constriction in the blood vessels supplying these centres and therefore that the cessation of activity of the respiratory centre caused by adrenalin was in all probability not due to vaso-constriction but to a direct action on the nerve cells of the centre.

The flaw in this argument is the assumption that because anæmia abolishes the action of the respiratory centre it must abolish or reduce the action of other centres. In my previous paper⁽³⁾ I have shown that this is not the case for complete occlusion of the arteries to the bulb, whilst stopping respiration, powerfully excites the vaso-motor and cardio-inhibitory centres.

Huggett and Mellanby bring forward a teleological explanation of the respiratory effects of adrenalin which is linked up with the "emergency hypothesis" of adrenalin. They believe that in time of stress the depressant action of adrenalin serves to check the hyperpnoea due to afferent impulses. This appears to me to be most unlikely for not only is there no evidence to show that under such circumstances of stress hyperpnoea tends to be excessive there is the more pertinent fact that the dose of adrenalin which is required to bring about an appreciable reduction in respiration is many times greater than the greatest amount which has been shown ever to exist under natural conditions in the blood.

In the experiments of Huggett and Mellanby the duration of adrenalin action was brief. Obviously, if complete anæmia, similarly brief, has no more effect on the bulbar reflexes than is produced by adrenalin, the argument of Huggett and Mellanby falls to the ground. I have therefore made observations on the effect of brief occlusion of the arteries supplying the bulb on the reflexes dealt with by these workers.

The experiments described below were performed on rabbits and cats anæsthetised with urethane supplemented by c.e. mixture. The records of respiration and blood-pressure and the occlusion of the bulbar arteries were carried out by the methods described in earlier papers.

The depressor reflex. For the investigation of this reflex rabbits were employed. During the complete anæmia produced by occlusion of the cerebral arteries the depressor reflex persists. In fact the slowing of the heart may be greater than that which is produced by the same strength of stimulation with the arteries open. An example of this is shown in Fig. 1. It will be seen that the depressor before anæmia caused a fall of pressure of 46 mm. (110 to 64) and during the apnoea and rise

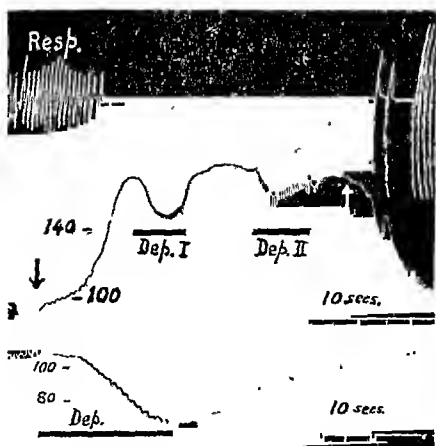


Fig. 1. Upper figure, stimulation of the depressor nerve during occlusion (between the arrows) of the cerebral arteries. Lower figure, stimulation of depressor with the same strength, the arteries being open.

of blood-pressure it caused a fall of 20-22 mm. (170 to 148 and 178 to 158). The rate of heart-beat, which was reduced by 24 per minute (288 to 264) by stimulation before anæmia, was reduced during the first depressor stimulation during anæmia by 108 (300 to 192) and still more reduced (to 144) as the anæmia continued (second stimulation).

This shows that in the rabbit when anæmia of the medulla alone does not greatly stimulate the cardio-inhibitory centre the threshold of this centre to inhibitory stimuli may be lowered. It shows further that the lowering of the threshold may become greater as the effects of arterial occlusion accumulate.

As stated by Huggett and Mellanby in order to demonstrate the persistence of the depressor reflex during adrenalin apnœa submaximal doses must be used, for if adrenalin is given in sufficient quantity to produce absolute apnœa the peripheral action of the drug prevents the manifestation of the reflex. But with doses sufficient to reduce respiration to slight movements of the epigastrium the reflex can be shown to persist. Here again the reduction in rate of beat may be increased as is shown in Fig. 2. Before adrenalin depressor stimulation reduced the heart-beat by 12 beats per minute (from 222 to 210). During the action of adrenalin stimulation with the same strength reduced it by 90 (from 222 to 132).

The vaso-motor centre. Huggett and Mellanby have shown that the rise in pressure produced by adrenalin can be diminished by depressor stimulation after section of the vagi and reinforced by stimulation of the femoral nerve. I have found corresponding effects during anæmia. Thus, in one experiment, after section of the vagi and during occlusion of the arteries, stimulation of the depressor caused a fall of blood-pressure from 163 to 140, while stimulation of the femoral nerve caused a rise from 160 to 190.

The swallowing reflex. This reflex was obtained by stimulation of the central end of the superior laryngeal nerve, movement of the larynx being recorded. It was found to persist during arterial occlusion (Fig. 3) and during adrenalin action.

The corneal reflex. The same is true of this reflex.

The oculo-motor reflex. In the case of this reflex the conditions of the experiment are not identical. With adrenalin the pupil is of course dilated by the peripheral action of the drug. Upon occlusion of the arteries the pupil first becomes contracted, indicating that anæmia stimulates the centre of the ciliary nerve as well as the vagus and vaso-motor centres. If the occlusion is allowed to persist the pupil dilates as it does in the moribund state from whatever cause. In spite of this

difference the pupil responds to light both during adrenalin action and in the early stages of arterial occlusion.

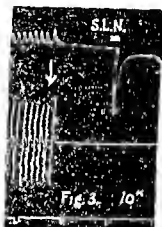
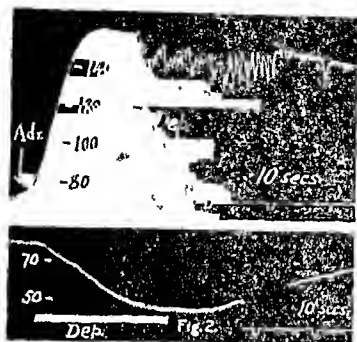


Fig. 2. Upper figure, depressor stimulation during the action of adrenalin (1 c.c. of 0.01 p.c. solution intravenously) Lower figure, depressor stimulation with the same strength without adrenalin. Compare with Fig. 1.

Fig. 3. Persistence of the swallowing reflex during arterial occlusion (at the arrow). The upper tracing is movement of the larynx, movement upward being recorded as a downstroke. The lower tracing is of respiration from the trachea tube S.L.N., stimulation of superior laryngeal nerve.

Fig. 4. Suppression of excitability of the respiratory centre to femoral nerve stimulation upon arterial occlusion (at the arrow).

The knee-jerk. In order to obtain anæmia of the lumbar region of the spinal cord the abdominal aorta was clamped at its origin, that is, above the point at which the celiac axis arises. The knee-jerk was found

to persist during periods of occlusion lasting up to one or two minutes. That such occlusion causes a sufficiently severe anæmia is shown by two facts. First, the pressure in the aorta below the clamp is zero, indicating that the collateral circulation by way of the spinal arteries is very feeble. Secondly, if the clamp is allowed to remain for a longer period, say about 10 minutes, the knee-jerk disappears.

The respiratory centre. Huggett and Mellanby have shown that the reflex excitability of the respiratory centre as tested by stimulation of the femoral nerve is suppressed when the centre is under the influence of adrenalin. In Fig. 4 is seen the effect upon respiration of occlusion of the cerebral arteries during stimulation of the femoral nerve. It will be seen that the hyperpnœa due to stimulation alone is first intensified and then gives way to almost complete apnœa.

Discussion.

These experiments, while not deciding the question of the mode of action of adrenalin, show that the results obtained by Huggett and Mellanby are not opposed to the vaso-constrictor theory. The general similarity between the effects of adrenalin and those of brief anæmia is, as far as it goes, in favour of the theory. It accords with the evidence already brought forward. There is first the fact which I have shown⁽⁴⁾ that all the most important vaso-constrictor substances have the same effect upon respiration, a fact which on the hypothesis of direct paralysis would, to my mind, be a very extraordinary coincidence. Secondly, it has been demonstrated by Huggett and Mellanby (though denied, it is true, by Bouckaert⁽⁵⁾) that the respiratory effect is annulled when the vaso-constrictor action of adrenalin has been abolished by ergotoxin. Thirdly, in the Cheyne-Stokes respiration, which often follows the apnœa, there occur, synchronously with the respiratory periods, fluctuations in the Circle of Willis pressure, which seem to indicate very strongly that the blood vessels arising from the Circle are undergoing rhythmic contraction and relaxation⁽⁶⁾. These facts constitute, I think, strong though indirect evidence for vaso-constriction.

SUMMARY.

1. The reflex excitability of various nervous centres, when deprived of their blood-supply for short periods, has been investigated and compared with the reflex excitability during the action of adrenalin.
2. It has been found that under both conditions the pressor, depressor,

corneal, oculo-motor and swallowing reflexes and the knee-jerk persist while the cardio-inhibitory centre may become more excitable.

3. Under both conditions the excitability of the respiratory centre to afferent stimuli is suppressed.

4. The close similarity in the behaviour of the centres under the two conditions is evidence in favour of the vaso-constrictor hypothesis of adrenalin apnoea.

The expenses of the above research have been partly defrayed by a grant from the Government Grant Committee, Royal Society.

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THE INFLUENCE OF THE VAGUS ON THE ISLETS
OF LANGERHANS. Part I. Vagus hypoglycæmia.
By G. A. CLARK.

*(From the Physiological Laboratories of the Durham University
College of Medicine and Sheffield University.)*

ALLEN(1) has shown that sugar tolerance in the dog is not lowered when a sufficient pancreas-graft in the spleen is separated from all original connections other than its duct, while investigations of Banting and Gairns(2) also indicate that the secretion of insulin is not necessarily dependent on the nerve supply to the islets of Langerhans and is normally a continuous process. Nevertheless, it is not unreasonable to suppose that the nervous system may influence the secretion of insulin. The general physiological antagonism between sympathetic and parasympathetic systems suggests the possibility of some mechanism controlled by the parasympathetic to counteract the hyperglycæmia produced by sympathetic activity. Eppinger and Hess state that pilocarpine can arrest adrenaline hyperglycæmia(3) and that atropine in certain cases may cause glycosuria(4). These phenomena are explicable if it can be shown that stimulation of the parasympathetic will produce an increase of insulin in the blood. The nerve-supply to the islets of Langerhans is conveyed mainly, if not entirely, in the right vagus and Macleod and his co-workers(5) have produced hypoglycæmia by stimulation of the right vagus in the neck. Indirect evidence of a nervous factor causing hypoglycæmia was given in a recent paper(6), where it was shown that guanidine will produce in rabbits an immediate fall in blood-sugar if sympathetic glycogenolysis is prevented by ergotoxine, or if the animal's glycogen store has been previously depleted. This hypoglycæmic action was found to be inhibited by atropine. In the present paper more direct evidence is offered of the influence of the vagus on the secretion of insulin.

As in the previous investigation(6), Bang's old micro-method for the estimation of blood-sugar was used, blood being obtained from an ear vein. The normal blood-sugar content was determined immediately before an experiment by three estimations at intervals of half an hour.

All drugs were administered intravenously and food was withheld from an animal for 18 hours before any experiment

Effect of pilocarpine In view of the effects of guanidine referred to above, it was anticipated that any drug stimulating the parasympathetic would, under suitable conditions, produce a lowering of blood sugar. Each of three rabbits was therefore given 3 mgms of ergotamine tartrate followed one hour later by pilocarpine nitrate in .5 p c solution, No 1 received 5 mgm per kilo, No 2, .75 mgm per kilo, and No 3, 1 mgm per kilo. In all three cases a fall in blood sugar resulted, that in No 3 being approximately of the same order as that produced by .5 gm guanidine hydrochloride per kilo (Table I). It has recently been stated that ergotamine alone will lower the blood sugar of normal rabbits (7). In numerous control experiments I have found no change in blood-sugar level within an hour of the intravenous injection of 3 mgms ergotamine tartrate and in two cases in which estimations were made over a period of five hours after ergotamine alone the following values were found

Hours after injection	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$	4	4 $\frac{1}{2}$	5
	124	122	—	120	120	—	118	118	—	116	—
	120	—	124	—	128	—	118	116	114	—	120

It is evident then that ergotamine cannot be responsible for the immediate fall in blood sugar when this drug precedes the administration of pilocarpine or guanidine. This is also shown by the fact that pilocarpine alone in doses of 1 mgm per kilo lowers blood sugar (Table I).

TABLE I

Pilocarpine

Hours after injection	Guanidine 5 gm per kilo	After ergotamine			Alone	
		3	2	1	4	5
0	120	105	110	124	120	120
$\frac{1}{2}$	112	090	097	110	100	100
1	096	083	090	102	086	092
$1\frac{1}{2}$	090	080	085	105	092	110
2	092	085	—	110	107	115
$2\frac{1}{2}$	105	—	096	—	109	—
3	110	100	102	124	—	122

Guanidine after vagotomy Direct evidence of the influence of the vagus in producing hypoglycaemia was sought by investigating the effect of guanidine hydrochloride on the blood sugar in rabbits in which this nerve was divided in the neck. The operation was performed on the right vagus in six animals, the hypoglycaemic action of guanidine having previously been determined in two of them. (It may here be recalled that in an intact rabbit a second dose of guanidine preceded by ergota-

mine almost invariably produces a greater fall in blood-sugar than the first, even when an interval of several weeks intervenes between the doses(6.) Two weeks after section of the vagus in the case of No. 4 and No. 5, three in the case of No. 6 and No. 7, four in the case of No. 8 and five in the case of No. 9, 3 mgms. ergotamine tartrate were given, followed one hour later by .1 gm. guanidine hydrochloride per kilo. It will be seen that in one case only did hypoglycæmia result (Table II).

TABLE II.

Hrs after injection	Before vagotomy		After vagotomy (right side)					
	4	5	4	5	6	7	8	9
0	.122	.112	.125	.132	.115	.117	.110	.125
$\frac{1}{2}$.091	.085	.122	.128	.120	.115	.108	.120
1	.076	.068	.124	.136	.110	.115	.105	.101
$1\frac{1}{2}$.085	.075	.128	.134	.121	.117	.100	.074
2	.095	.095	.124	.130	.132	—	—	.076
$2\frac{1}{2}$	—	—	—	—	.128	.125	.110	—
3	.110	.105	.118	.125	.125	—	—	.084
$3\frac{1}{2}$	—	—	—	—	—	.127	.110	—
4	.116	.114	.128	.125	.115	—	—	.090

In two animals the left vagus was resected in the neck, the normal reaction of the blood-sugar to guanidine having been previously determined in one of them. Two weeks after the operation in one case and three weeks in the other .1 gm. per kilo of guanidine hydrochloride, preceded by 3 mgms. ergotamine, was injected, with the result that one rabbit showed no alteration in blood-sugar while a hypoglycæmia occurred in the other as shown below.

Hrs after injection		0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3
Before vagotomy No. 10		.115	—	.085	.080	.090	.103	—
After " " 10		.100	.077	.070	.068	.080	.090	—
" " 11		.116	.110	.116	.114	—	—	.120

Guanidine has been shown to stimulate the preganglionic fibres of the vagus(8), and the above results may thus be interpreted as indicating that in the majority of rabbits the right vagus in the neck carries fibres, stimulation of which causes a lowering of blood-sugar, but in some cases these fibres appear to be carried in the left vagus.

Guanidine and "Infundin." That increased liberation of insulin is responsible for the hypoglycæmia produced by vagal stimulation is suggested by two experiments based on the observation of Burn that pituitary extract diminishes or abolishes the hypoglycæmia normally brought about by insulin(9). Each of two rabbits was given a preliminary injection of ergotamine, followed one hour later by .1 gm. guanidine hydrochloride and 1 c.c. "Infundin" (Burroughs, Wellcome and Co.) per

kilo. It is seen from Table III that although "Infundin" alone and guanidine alone (ergotamine having been previously given in each case) cause a definite fall in blood-sugar, the same amounts of each given simultaneously have much less effect on the blood-sugar in one rabbit and practically no effect in the other.

TABLE III.

Hrs after injection	"Infundin"		Guanidine		Guanidine and "Infundin"	
	No. 3	No. 12	No. 13	No. 3	No. 13	No. 3
0	·105	·110	·110	·120	·110	·120
$\frac{1}{2}$	·085	·104	·080	·095	·105	·118
1	·065	·092	·070	·080	·100	·110
$1\frac{1}{2}$	·070	·075	·065	·082	·094	·112
2	·075	·070	—	—	·090	—
$2\frac{1}{2}$	—	—	·070	—	—	·112
3	·080	·075	·075	—	·105	—
$3\frac{1}{2}$	—	—	—	·085	·110	·117
4	·085	—	·090	·095	·105	—

It is of interest to note that the mixture of guanidine and "Infundin" appeared to be particularly toxic in the case of No. 12 (an old rabbit) which after a preliminary few moments of intense excitement became comatose and was killed. The youngest of the three (No. 3) showed no untoward symptoms, while No. 13 after a few minutes of mild excitement appeared normal. It may seem possible that the antagonism shown in these experiments is one between pituitary extract and guanidine and not pituitary extract and insulin, but in view of the symptoms shown by the animals this is improbable, and in the absence of blood-sugar estimations, mere observation would have suggested that "Infundin" facilitated guanidine intoxication.

Glucose tolerance after vagotomy. As a sequence of the foregoing results, the question naturally arose whether the vagus normally exerts any control on the sugar content of the blood. If it does, the glucose tolerance of animals might be expected to be altered after vagotomy. In Table IV are given blood-sugar values found at half-hourly intervals following the intravenous injection of 1 gm. of pure glucose per kilo of body-

TABLE IV.

Hrs after injection	Before vagotomy			After vagotomy		
	No. 14	No. 15	No. 16	No. 14	No. 15	No. 16
0	·120	·122	·122	·120	·122	·126
$\frac{1}{2}$	·315	·305	·315	·282	·285	·264
1	·164	·178	·225	·214	·145	·122
$1\frac{1}{2}$	·137	·150	·116	·125	·125	·124
2	·118	·125	·122	·116	·122	·120
$2\frac{1}{2}$	—	—	—	·120	—	—

weight in rabbits before and two weeks after section of the right vagus in the neck. The glucose was given in 10 p.c. solution at the rate of 10 c.c. in 3 minutes.

The lower sugar value found half an hour after injection in the animals after vagotomy compared with the corresponding observation before vagotomy is apparently of no significance, as a similar phenomenon was observed in a second tolerance test on normal rabbits. It is evident then that this test gives no indication of altered sugar tolerance after vagotomy. Similarly, no alteration was found in three rabbits that had been given .2 mgm. atropine sulphate per kilo 15 minutes before the sugar injection. These results were to be expected in view of the findings of Allen⁽¹⁾ and of Banting and Gairns⁽²⁾.

Discussion.

The significance of the nerve supply to the islets of Langerhans is obscure. That a ready supply of insulin is necessary to deal with a sudden increase in blood-sugar is apparent in that continued hyperglycæmia can produce atrophy of islet tissue, and again, in the absence of islet tissue hyperglycæmia is practically limited by the relation of excretion to production and ingestion of glucose. All available evidence indicates that increase in blood-sugar is itself a sufficient stimulus for the production of insulin. It is possible that the action of the vagus on the islet cells is merely secondary to alteration of the blood supply. Banting and Gairns⁽²⁾ have shown that increase in blood-flow to the pancreas causes an increase in the insulin output. If vagal hypoglycæmia is due to vasodilatation in the pancreas some difference might be expected in the action of pilocarpine in lowering blood-sugar when given alone and when the sympathetic system is depressed by ergotamine. Table I, however, indicates no obvious difference. It is difficult to believe that the vagus supply to the islets is functionless and the possibility must be considered that the influence is similar to that of the fibres supplying the acinar cells. Mellanby⁽¹⁰⁾ has shown that when the enzyme content of pancreatic juice, stimulated to flow by secretin, has fallen to a low level it may be raised to its original value by vagal stimulation, which appears to facilitate the manufacture of enzymes by the acinar cells. If the vagus supply to the islets has a similar function it ought to be easier to exhaust the islets in an animal with the vagus cut than in a normal animal. The glucose tolerance test described above gives no indication of this, but tends to show that sufficient insulin can be produced to meet an emergency without vagal control. This aspect of

the problem is at present the object of further investigation. Whatever may be the mode of action of the vagus in the foregoing experiments, the results suggest that caution may be necessary in the administration to diabetics or potential diabetics of drugs which stimulate the vagus.

SUMMARY.

In the rabbit, drugs which stimulate the parasympathetic system cause a lowering of blood-sugar under the conditions described. This effect is not produced after section of the right vagus in the great majority of rabbits; it may be prevented in some animals by section of the left vagus.

The results described suggest that stimulation of the vagus causes a secretion of insulin.

I wish to express my thanks to Prof. Burns and to Prof. Leathes for much helpful criticism.

The expenses for part of this work were defrayed by a grant from the Medical Research Council to the Department of Physiology of Durham University.

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FURTHER OBSERVATIONS ON NUTRITION WITH DIETS RICH IN PROTEIN. BY V. B. READER AND J. C. DRUMMOND.

(From the Biochemical Department, University College, London.)

IN spite of considerable study of the influence of diets rich in protein on growth and nutrition it remains uncertain whether they are adversely affected or not. Obviously in species which are carnivorous normal nutrition can be maintained when the food contains a large proportion of protein, but in omnivorous and herbivorous animals abnormal conditions have from time to time been ascribed to excessive nitrogen intake. Drummond, Crowden and Hill(1) found that rats fed on diets containing from 80-90 p.c. of the dry weight as protein (caseinogen) exhibited a subnormal rate of growth and failed to reach adult size. Post-mortem examination of the animals revealed no marked lesions other than a slight beading of the rib junctions; nor did the histological study of the tissues show abnormal changes. In particular, the kidneys, in spite of a relatively enormous daily excretion of nitrogen, appeared normal as compared with the controls and showed none of the degenerative changes observed in rabbits by Newburgh(2).

In 1923 Polvogt, McCollum and Simmonds(3) recorded experiments on rats fed on diet containing from 30-40 p.c. of protein. Their animals grew at a normal rate and reproduced satisfactorily for several generations, but usually showed evidence of kidney lesions in spite of the fact that their diet cannot be regarded as having been very abnormally rich in protein. More recently Osborne and Mendel(4) have claimed that normal growth to adult size may be obtained in rats fed on diets in which all carbohydrate and fat is furnished endogenously. They record hypertrophy of the kidneys but could detect no structural damage.

In view of these discrepancies a further series of experiments have been made.

Young rats weighing about 50 gms. were fed on diets compounded as follows:

	Group I	Group II	Group III
Caseinogen	20 parts	45 parts	90 parts
Starch	70 "	45 "	0 "
Cod liver oil	2 "	2 "	2 "
Yeast extract	5 "	5 "	5 "
Lemon juice	5 "	5 "	5 "
Salt mixture	5 "	5 "	5 "

Occasionally there was an initial drop in weight, especially in Group III, if the rats were less than 50 gms. in weight when first placed on the diet and a few rats died. The majority recovered and grew.

Figs. 1 and 2 show typical growth curves of the three groups of rats when fed on these diets.

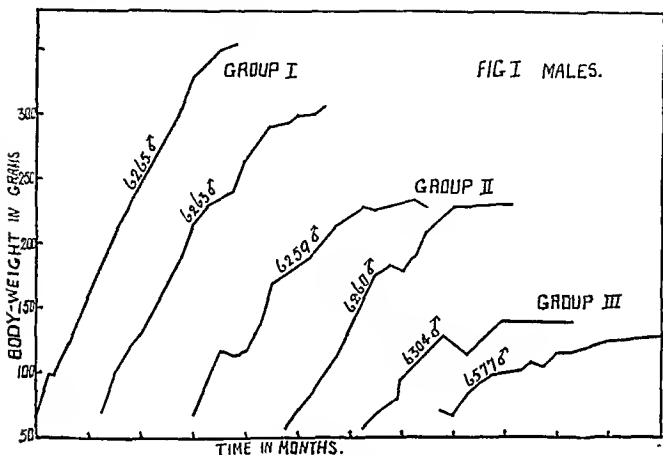


Fig. 1. Males.

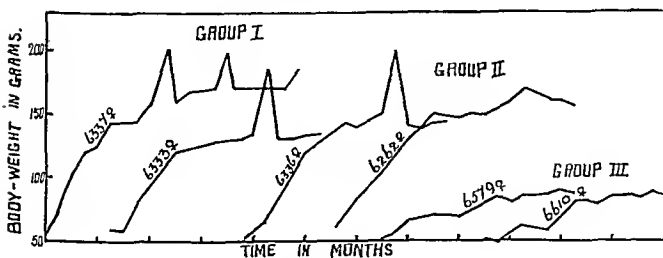


Fig. 2. Females¹.

The rats in Group I showed normal growth, as was to be expected. Those in Group II at first showed a similar behaviour, but later a retarda-

¹ The sharp peaks in these curves indicate pregnancies.

tion of growth set in and they failed to reach full weight. The animals in Group III failed to show normal growth at any stage and attained a constant weight of approximately one-third the calculated normal weight. Throughout the experiment the animals in all three groups were quite healthy and presented a satisfactory appearance, apart from the cases of stunting to which reference has already been made. Records of food consumption over periods of ten days were made several times during the experiments, and revealed the fact that more food is eaten when the percentage of protein in the diet is raised. This may be seen from the figures given below:

Group	% protein in diet	Average weight of rat	Daily con- sumption of dry food in gms.	Consumption per 100 gms. body weight
I	20	100	10.5	10.5
II	45	100	12.7	12.7
III	90	75	17.9	23.9

In view of the earlier experiments in which it was suspected that there was interference with ossification in the animals receiving much protein, periodic X-ray examination was made of the rats in the three groups in this experiment. Careful study of the radiographs disclosed no abnormalities and it is concluded that calcification had proceeded normally even in the stunted animals on diet III. This was later confirmed by histological examination of the costochondral junctions in a number of cases.

The experiments were continued sufficiently long for reproduction to take place, but while Group I produced many litters, and Group II a few, there was no reproduction in Group III.

A number of animals were killed after they had been about four months on their respective diets, and were subjected to careful examination. The organs presented a normal appearance; there was plenty of body-fat and apart from the small size of the animals in Groups II and III they might all have been normal rats. In the earlier experiments made in this Laboratory it appeared that the kidneys of the animals on the protein-rich diet were enlarged, but insufficient were then examined to justify a statement. Osborne and Mendel⁽⁴⁾ found the average weight of the kidneys on the protein-rich diet to be almost twice that of the kidneys of control animals, whilst their size was about one-third greater. We have now obtained ample confirmation of this hypertrophy.

The following figures give the ratio of kidney weight to body weight for a number of rats of the same age from each group:

I (20 % protein)	II (45 % protein)	III (90 % protein)
·0063	·0103	·0163
·0069	·0099	·0149
·0079	·0102	·0150
·0090	·0094	·0196
·0079	·0101	·0237
·0084	·0104	·0174
·0090	·0098	·0155

Donaldson(5) gives the normal range for this age as ·0070–0090. Histological examination was made of the livers and kidneys, using Zenker's fixative and staining with hæmatoxylin and eosin, but no abnormalities were detected which could be ascribed to the diet.

DISCUSSION.

We are unable at this stage of the enquiry to offer an explanation of the failure of the rats to grow normally on the diets containing 45 and 90 p.c. of protein. Both Polvogt, McCollum and Simmonds(3) and Osborne and Mendel(4) obtained better growth than we. In the former case their diets never contained more than 41 p.c. of protein and therefore may reasonably be compared with our Group II, but there are differences between the rations employed for we used only one protein, caseinogen, whilst they used mixtures of caseinogen with the proteins present in such natural foods as wheat, maize, navy beans and liver. On the other hand, Osborne and Mendel observed better growth on a diet containing 50–55 p.c. of caseinogen, together with some fat and carbohydrate, than we recorded on the ration containing 45 p.c. caseinogen. There appear to be no experiments strictly comparable with ours in which 90 p.c. of caseinogen was used. In view of the different types of diets employed by the various investigators it is almost impossible without further experimentation to suggest the cause of the discrepancies, but it is just possible that the recent work of Hartwell(6) may throw some light on the matter. She has traced what she believes to be a relation between the amount of vitamin B required in the food and the protein content of the diet. As yet the evidence appears insufficient to prove this relation, but if further work should establish that it does exist it may provide an explanation of the discordant results we have outlined.

An admittedly inadequate attempt to test whether her theory is correct was made by administering extra amounts of yeast extract to a few rats the growth of which had been retarded by the protein-rich diets. Sometimes there was a slight response but the results were inconclusive and call for further work. In a few other cases rats from the

same group were given an extra supplement of cod liver oil in case a larger amount of the vitamins in that foodstuff was necessary, but no acceleration of growth took place. When rats from Groups II and III were given the ration of Group I there was usually a resumption of growth, sometimes at a nearly normal rate, but before long it ceased and the animals failed to reach a normal size. (See Fig. 3.) Here again we offer no explanation, preferring to wait for the results of further experiments.

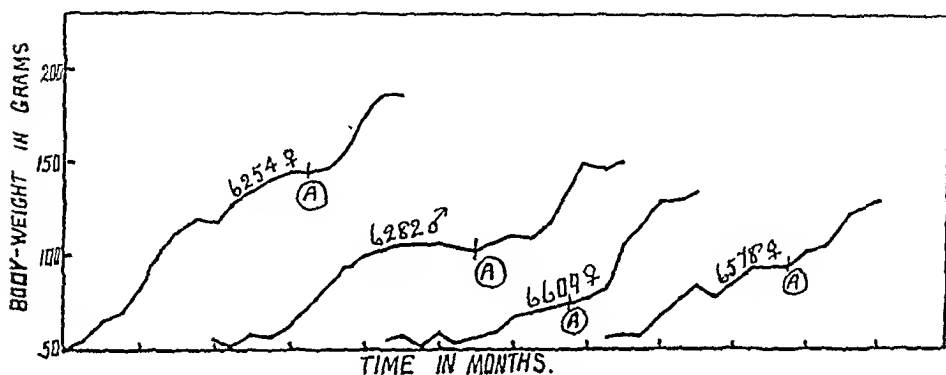


Fig. 3. Group III changed to Group I diet at (A).

The hypertrophy of the kidneys, which we observed, doubtless results from the increased work which the kidneys are called upon to perform when so large an amount of nitrogenous waste products must be excreted. It has been frequently stated, largely from clinical observation, that kidneys may become enlarged from this cause, and it has also been repeatedly denied. These results, confirming those of Osborne and Mendel, definitely prove that physiological hypertrophy may occur. It has also been widely held that the prolonged excretion of abnormally large amounts of nitrogenous waste products will induce degenerative changes in the kidneys and no small part of the literature on Bright's disease concerns this point. Here again, this view has been established largely on the results of clinical observation and has lacked controlled experimental support.

What at one time appeared to be adequate support for this widely held opinion was furnished by the paper of Newburgh(7), who stated that rabbits fed on diets rich in protein almost invariably show, after a few weeks, marked nephritis. Careful examination of his experimental records, however, tends to shake one's confidence in his conclusions. Many of his animals were fed on ill-balanced and inadequate rations, such as a diet consisting solely of egg-white, which might, and probably

would, produce pathological changes. Furthermore, many of the kidneys examined were removed from the body after the animals had been found dead from inanition or as a result of the diet. This is not justifiable. Certainly other experiments of his are less open to severe criticism and if the results in these cases are reliable it would indicate that the rabbit may show kidney damage as a consequence of feeding on diets rich in protein.

It is less easy to understand why degenerative changes in the kidneys of rats were observed by Polvogt, McCollum and Simmonds(3) when their animals were fed on well-balanced diets containing the relatively small proportion (30-40 p.c.) of protein. As a matter of fact they state that the characteristic picture contrasted with that observed in the kidneys of control rats was one of enlargement and congestion. In some cases there was degeneration of the tubular epithelium and hyaline casts were present. The enlargement we have already considered. Some congestion was noted in practically all the kidneys we examined, the differences between the three groups being insignificant. The degeneration may possibly be due to the longer period that their animals were kept on a protein-rich diet, but we do not ourselves think this is the explanation. Slight abnormalities of the type they describe are, we believe, fairly common in rats, and may possibly be associated with the many minor ailments to which they are subject, particularly from internal or external parasites, and about which little or nothing is known. In our rats one or two cases showed extremely slight degeneration of tubules with occasionally small patches of leucocytic infiltration. These abnormalities were observed, however, as frequently in the control group as in the groups receiving large amounts of protein, and we, therefore, cannot agree that the excretion of abnormally large amounts of nitrogenous end-products of metabolism causes damage to the kidney in the rat.

SUMMARY.

1. Rats failed to grow to adult size when fed on diets containing a high proportion of protein (45-90 p.c.).
2. The animals appeared to be in good health and the consumption of food was satisfactory. Post-mortem examination revealed no abnormality other than hypertrophy of the kidneys, as measured by the ratio of kidney weight to body weight.
3. The slight degenerative changes which were observed in some of the kidneys of rats fed on the diets rich in protein could not be ascribed

to the action of the food as similar changes were seen in the controls. Excretion of relatively very large amounts of nitrogen (as much as 2.5 gm. daily) over periods of four months does not appear to damage the kidneys of rats.

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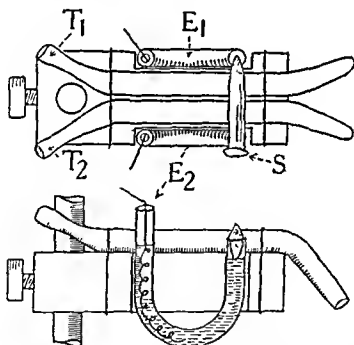
PHYSIOLOGICAL SOCIETY,

May 24, 1924.

A class experiment on the nature of the injury current.

By E. D. ADRIAN.

According to the theory developed by Bernstein the potential differences which give rise to the action current and the injury current of a muscle or nerve fibre are due to inequalities in the distribution of various ions within and without the fibre. These inequalities are supposed to be present in the resting, uninjured fibre, and they are prevented from dissipating themselves by membranes which have a selective permeability for the various ions. A local injury causes the current by breaking down the restraining membranes so that a movement of ions takes place



through the damaged region, but the energy is derived, not from any chemical change taking place in this region, but from the state of strain existing at the intact membranes in other parts of the fibre.

According to this view we ought to find that the E.M.F. of the injury current will vary, like that of a concentration cell, directly with the absolute temperature. We ought also to find that the E.M.F. will not be altered by a change of temperature unless this affects the neighbourhood

of the electrode applied to the intact surface; a change of temperature in the injured region should have no effect because the latter merely plays the part of an indifferent conductor. Bernstein and others have shown that both these results are obtained over a limited range of temperature in frog's muscle and nerve, and the experiment is easily adapted for class purposes. A frog's sartorius S with the tibial end damaged by heat rests on two glass tubes T_1 and T_2 , through which cold or warm water can be passed. The tubes are fixed to the top of a hard wood block which carries on either side a non-polarisable electrode E_1E_2 , held in position with plasticene. The electrode is a small glass U-tube filled with Ringer and loosely plugged at one end with cotton wool. The end of the muscle rests on this cotton wool and the current is led off by a coil of silver wire coated with silver chloride dipping into the other limb of the U. The E.M.F. due to the muscle is measured by balancing against a potentiometer wire by the aid of a mirror galvanometer and universal shunt. The experiment is divided into two parts: in the first, the two tubes T_1T_2 are connected to the same reservoir and the E.M.F. is measured whilst water at various temperatures is passing through them. It is assumed that the temperature of the muscle is the same as that of the outflowing water, and with this assumption the E.M.F. is found to be roughly proportional to the absolute temperature. In the second part of the experiment the two tubes are maintained at different temperatures and it is found that the E.M.F. does not depend on the temperature of the tube under the damaged (tibial) end of the muscle but only on that of the other tube.

The arrangement has been used as an advanced class experiment for the past four years and gives consistent results. Various controls for testing the electrodes, etc. are easily carried out and the same stand can be used for other experiments on the injury current (effect of ions, E.M.F. of muscles in series, etc.). It might be an improvement to substitute varnished metal tubes of square cross-section for the glass tubes at present in use.

A method for investigating the condition of the nasal mucous membrane. By A. J. COPELAND.

Nasal mucous membrane has almost the structure of erectile tissue, and investigation of the nervous control of its vascularity and also of the changes produced by the direct application of local anæsthetics and other drugs is of importance. Such experimental investigation has hitherto

presented difficulties. The narrow orifice of the external nares of the rabbit, cat and dog precludes direct observation of the mucous membrane excepting after such an operation as would alter the natural condition of the part. To obviate this difficulty a method for obtaining such information indirectly has been devised, and has given reliable results in a number of experiments.

In both cat and dog there is no foramen between the two frontal sinuses, both of which communicate by small separate foramina with their respective nasal fossæ. By injecting into one of these frontal sinuses a watery solution of methylene blue it was found that fluids introduced into one of these sinuses and allowed to flow through the nares come into contact with the whole of the mucous membrane of both nasal cavities. This Schneiderian or pituitary membrane, which closely invests the outer walls and meatuses of the fossæ, is thick and vascular, as in man, and large venous plexuses are surrounded by bands of plain muscle fibres to form a kind of cavernous tissue. The special olfactory mucous membrane which lines the upper part of each cavity is peculiarly vascular, and contains a close plexus of large capillary vessels.

Both dog and cat are suitable for the following experiments. The method consists in allowing fluid at constant temperature and pressure to enter a frontal sinus, and then measuring its rate of flow as it drips from the nose. The animal is anæsthetised with ether, and then with urethane or chloralose (solid anæsthetics which do not affect vessels), and breathes through a tracheotomy tube. One of the frontal sinuses is opened with a small trephine, and into the opening a small brass tube is screwed, to which is attached the rubber outflow tube of a Marriotte's flask filled with saline at body temperature. Fluid is now allowed to enter the sinus, and the flask is fixed at a height of about 2 inches above the brass tube, so that there is a fast drip of fluid from the nose. The outflow is collected in a graduated flask, which is replaced at the end of periods of 15 or 30 seconds, as measured with a stop watch. At the beginning of the perfusion the rate of flow becomes augmented, but it is soon constant. The flow is now stopped by clipping the rubber tube near its end, and a small quantity of the solution of the drug is slowly injected through the rubber tube into the frontal sinus, from which it flows into the nares to fill up both nasal cavities. The drug is allowed to remain in contact with the nasal mucous membrane for a given time, after which perfusion is repeated as before. Swelling of the mucosa will increase the resistance and diminish the rate of flow: conversely constriction of vessels is shown by an augmented flow.

A few typical results are given:

1. *Stimulation of sympathetic nerves.*

Stimulation of one or both cervical sympathetic nerves by the induction coil always caused immediately marked augmentation of the flow.

In an experiment on a cat the rate of flow for consecutive periods of 15 seconds = 9 c.c., 8.8 c.c., 8.4 c.c., 8 c.c., 8.5 c.c., 8.8 c.c., 9 c.c.

Both cervical sympathetic nerves were stimulated for 15 seconds; coil 10.

During stimulation = 15 c.c. in 15 seconds.

After stimulation = 19.5 c.c., 15 c.c., 13.5 c.c., 12 c.c., 11 c.c.

Here the maximum increase in the rate of flow was 116.6 p.c.

2. *Adrenalin.*

Adrenalin 1 in 10,000 causes some augmentation of the rate of flow.

In a cat the rate of flow in consecutive periods of 30 seconds = 6.5 c.c., 6.5 c.c., 7 c.c., 8.8 c.c., 9 c.c., 10 c.c. Fifteen minutes after 6 c.c. of 1 in 10,000 adrenalin rate = 7 c.c., 9 c.c., 10 c.c., 11 c.c., 12.2 c.c., 12.5 c.c., 13.5 c.c., 14.5 c.c., 14 c.c.

3. *Cocaine.*

Cocaine in 5 p.c. solution causes vaso-constriction and consequently an increase in the rate of flow.

In a cat the rate of flow in consecutive 15 second periods = 9.5 c.c., 9.5 c.c., 9.5 c.c., 9.5 c.c., 9.2 c.c. Twenty-five minutes after 4 c.c. 5 p.c. cocaine the rate = 8.4 c.c., 9 c.c., 10 c.c., 10.5 c.c., 11.6 c.c., 12 c.c., 12.4 c.c., 12.5 c.c., 12.4 c.c.

4. *Butyn.*

Butyn invariably caused so much swelling of the mucous membrane that the fluid almost ceased to run, the congestion lasting for more than 40 minutes.

In a cat the normal rate in consecutive 30 second periods = 19.5 c.c., 19 c.c., 19.5 c.c., 19 c.c., 19 c.c.

Fifteen minutes after 6 c.c. of 5 p.c. butyn there was no flow until the pressure was raised by squeezing the rubber tube, after which the rate = 1.5 c.c., 1 c.c., 1 c.c., 0.8 c.c., 0.5 c.c., 0.8 c.c.

Metabolism in diabetes. By L. LAWN and C. G. L. WOLF.

The respiratory exchange in four cases of severe diabetes has been estimated at 30-minute intervals during a period of about three hours, immediately before and following the ingestion of carbohydrate, protein and fat, and a mixed meal containing each of these components. The experiments were performed with a view to throwing some light upon the immediate effect of insulin upon the metabolism of the diabetic. Blood sugar estimations and urinary analyses for glucose and acetone compounds were included in the experiments. These experiments appear to show:

(1) That the injection of insulin into a diabetic patient in the fasting state immediately produces an increase in the proportion of carbohydrate catabolised, as evidenced by a rise in the respiratory quotient.

(2) Coincident with this there is, in some cases, a depression of the total metabolism, dependent chiefly upon the amount of insulin injected.

(3) An injection of insulin half an hour before the ingestion of food tends to depress the total catabolism of that food, as shown by a comparison with the rise experienced when the same food without insulin is administered.

(4) In the majority of experiments insulin does not, within $2\frac{1}{2}$ to 3 hours after injection, increase the proportion of carbohydrate which is oxidised after the ingestion of food.

(5) There is a depression of the R.Q. immediately after food which is not influenced by insulin.

Effect of respiration on pulse wave velocity.

By SYLVIA K. HICKSON¹ and B. A. MCSWINEY.

In measuring records of pulse wave velocity as estimated by the hot wire sphygmograph, a certain variation in the time intervals has always been noted, which was thought possibly to be related to the respiratory variations in blood-pressure.

To investigate this point, the blood-pressure was recorded and carotid radial tracings were taken by means of the hot wire sphygmograph (1), together with a respiratory curve, from a normal subject seated comfortably in a chair. Long records were taken by means of a paper camera, and the records measured by the Lucas Comparator, or by means of a glass slide etched in millimetres.

¹ Working for the Medical Research Council.

It was found, on taking similar points on the inspiratory curve, that the carotid radial intervals varied very little, and seldom by more than 1 in 500. Similar points on the expiratory curve also varied very little from each other, but were found to differ from the inspiratory intervals by a greater amount, viz. 1 in 250. In some cases the variation was small, but in cases where it was marked a difference in blood-pressure during inspiration and expiration could be readily determined by the auscultatory method.

Table.

(TIME INTERVALS IN SECONDS.)

Subject	Inspiration	Variation	Expiration	Variation	Difference insp. and exp.
E. P. 171	0.1100	0.0048	0.0930	—	0.0170
(a)	0.1089				
	0.1052				
(b)	0.1012		0.0920		
	0.1000	0.0012	0.0950	0.0055	0.0092
	0.1000		0.0975		
E. P. 176	0.0806		0.0777		
(a)	0.0799	0.0018	0.0767	0.0017	0.0046
	0.0794		0.0760		
(b)	0.0856		0.0735		
	0.0850	0.0024	0.0742	0.0011	0.0121
	0.0832		0.0746		
E. P. 175	0.0879		0.0830		
(a)	0.0890	0.0013	0.0820	0.0010	0.0072
	0.0892		0.0826		
(b)	0.0982		0.0910		
	—	—	0.0927	0.0019	0.0072
	—		0.0929		
E. P. 173	0.0987		0.0951		
(a)	0.0988	0.0007	0.0950	0.0001	0.0044
	0.0994		0.0951		
(b)	0.0976		0.0964		
	0.0976	—	0.0964	0.0007	0.0019
	0.0976		0.0967		

The blood-pressure was found to be higher on expiration than inspiration in all cases, and this corresponded with the slightly increased time interval, and accounted for the lower pulse wave velocity during inspiration.

From the above table it will be seen that the variation between the maximum variation points on the inspiratory and expiratory curves is always greater than the maximum variation between two points taken from the same parts of the curve.

The importance of this appears to be that the accuracy of this method

is increased by estimating the velocity from time intervals of similar phases of respiration. Where short records only are taken, without a respiratory curve, the accuracy of the method is slightly decreased, as comparable points cannot be obtained.

The expenses of this research were defrayed by a grant from the Government Grant Committee of the Royal Society.

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The thresholds of functionally different fibres in a mixed nerve (uncut). (*Preliminary note.*) By JOHN FARQUHAR FULTON.

Gasser and Erlanger's discovery that the action current of the sciatic nerve of the frog is composed of at least three and probably four discrete waves travelling at different velocities¹, being separable from each other by means of their differing refractory phases, suggests the existence in a mixed nerve of fibres of different characteristics of excitability. In attempting to find such differences experimentally various methods are being employed, and the purpose of the present note is to record the results of stimulating the *uncut* sciatic nerve of the frog (winter *Rana temporaria*) with short break-induction shock tetani (70 per sec.). In general it was found that the lowest thresholds existed in fibres to muscles farthest from the spinal cord; the digital muscles, for instance, always responded before tibialis anticus, and tibialis anticus before gastrocnemius (see Table I), whether the stimuli were delivered in the lumbar region or in the thigh. The differences were more marked at low temperatures than at high. For determining the reflex threshold (which was usually about two-thirds higher than the motor threshold), the reflex contraction of the adductors was taken as an index, the electrodes being applied distal to their nerve supply. The condition of reflex excitability of these preparations was such that a slight tap on any of the digits evoked vigorous flexion. When the nerve was stimulated at an intensity well above the reflex threshold, visible central after-discharge could be seen in the responses of the adductors, while no trace of it could be observed in the recorded responses of gastrocnemius. It would appear from this that a rhythm of 70 per sec. rendered the motor cells in the anterior horn refractory to superadded central stimuli.

¹ *Amer. Journ. Physiol.* 63. 417-18 (1923); *ibid.* (in press) (1924).

TABLE I.

Showing the thresholds of fibres in the uncut sciatic nerve of a decerebrate frog; T. 12°; circulation vigorous in hind limb. Stimulated by 70 break-shocks per sec. for periods of 0.2 sec. (approx.). Secondary R. 20,000 ohm (graphite); coil coreless. Intensity gradually increased to the reflex threshold and then decreased.

Response	Cm. sec. coil	Berne units	Sec. constant
			(15 cm.) Primary R. varied
Digits flexed	26.6	60	10
" extended	26.0	—	—
Tibialis antic.	25.6	—	9
Gastroc. (just)	24.9	—	8
" (max.)	24.2	—	8
Reflex (adduct.)	22.3	105	5 to 6
Gastroc. (max.)	24.5	—	7
" (just)	25.0	—	8
Tibialis antic.	25.7	—	9
Digits	26.4	61	10

In addition to these observations it has been found on microscopic observation that induced shocks of the intensity used in these experiments did not stimulate the vasomotor nerves.

For reasons which will be stated in detail elsewhere, the lower excitability of the longest fibres is regarded as evidence of a higher conduction rate in these fibres—an adaptation which would make possible simultaneous stimulation of proximal and distal groups of muscles in a limb.

On the relation of thyroidectomy to the effect of insulin on the blood sugar of rabbits. By J. H. BURN and H. P. MARKS.

Various workers have previously stated that the effect of a given dose of adrenaline in producing hyperglycæmia or glycosuria is less in the absence of the thyroid gland. We have confirmed this by observations on four rabbits before and after extirpation of the thyroid gland. The demonstration by one of us that ergotoxine increases the magnitude and duration of the fall of blood sugar produced by insulin, suggested that in the normal animal the return of the blood sugar to the usual level involved the stimulation of the liver either by adrenaline, or by impulses passing down the splanchnic nerves. If this were so, the discharge of sugar from the liver during the action of insulin might be expected to be slower in the animal without its thyroid than in the normal. Since our experiments began, some evidence that thyroidectomised animals are more sensitive to insulin has been published by Bodanski and by Ducheneau, but neither observer compared effects on the same animals before and after thyroidectomy. Our observations, carried out on seven

rabbits, have consisted in a comparison of the results repeatedly obtained on each animal when normal with those obtained on the same animal after thyroidectomy, these arrive at the same result as do those of Bodansky and Ducheneau, but show in addition that rabbits relatively insensitive to insulin are much more affected in the direction stated by removal of the thyroid gland than are rabbits which are sensitive. The general inference may be drawn from our observations that the greatest cause of individual variations in the response of rabbits to insulin is variation in the activity of the thyroid gland. The following figures illustrate this.

Minimal convulsive dose

Rabbit	When normal	After thyroidectomy
1	Greater than 1.6 mgm per kgm	0.25 mgm per kgm
2	1.2 "	0.25 "
3	1.08 "	0.25 "
4	0.53 "	0.2 "
5	0.4 "	0.15 "
6	0.27 "	0.22 "
7	0.15 "	0.075 "

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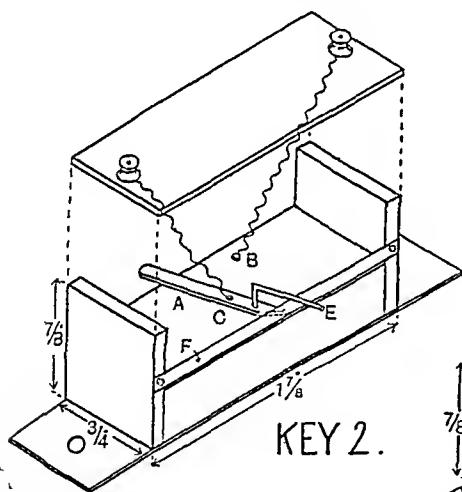
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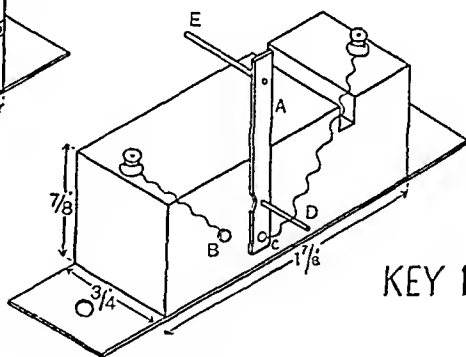
Two simple break keys. By S. L. MUCKLOW and B. A. McSWINEY.

The following two keys were designed with the primary object of providing a "Break" key which would be simple to make, would withstand regular use by students, and which would be interchangeable with the existing key on a Palmer drum.

Key 1. This consists of an ebonite block on which a light brass arm *A* is mounted so that it pivots about the screw *C*. In the "On" position the arm *A* makes contact with the stud *B*. Both *A* and *B* are connected



KEY 2.



KEY 1.

passing details on the ebonite block. A short rod *D* projects at right angles from arm *A* and this rod is struck by the revolving arm on the drum slower in the key. The rod *E* is merely for convenience in operation. experiments has been found very suitable where a slow moving drum is more sensitive

Ducheneau, b, second key was designed so that the contact would be before and after vibration, and is suitable for use with a fast-moving drum.

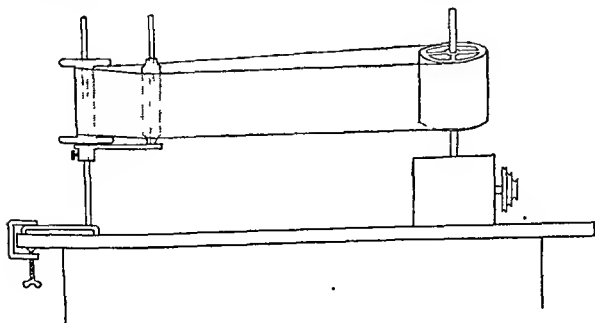
A brass arm *A* is mounted horizontally on an ebonite block so that it pivots about the screw *C*. One end of the arm *A* is fashioned into a cam which bears against a light watch spring *F*. In the "On" position the arm *A* makes contact with the stud *B*. Both *A* and *B* are connected to terminals on top of the key. The rod *E* is provided for convenience in operation. The key is operated by the revolving arm on the drum striking the arm *A*. It will be noticed that this key provides positive "On" and "Off" positions.

We are indebted to Mr F. S. Wilson, of the Physiological Department, Manchester University, for making up these keys to our designs.

Inexpensive long paper kymograph for student's use.

By WALTER J. DILLING.

The advantage of students in practical classes of a long paper kymograph is often apparent but the cost of providing such equipment is usually prohibitive. The simple arrangement indicated in the diagram is serviceable for both slow and fast speeds provided the paper travels horizontally. An ordinary stand, clamped to the bench, has adjusted on it a brass side arm with vertical steel rod. On the rod of the stand is placed a flanged hobbin of the width of the paper and on the rod of the side arm an unflanged hobbin to place tension on the paper and provide a flat writing surface between the two hobbins. A narrow flange on the lower rim of the driving drum prevents slipping from variation of paper tension. The cost of side arm for stand and of hobbins should not exceed five shillings.

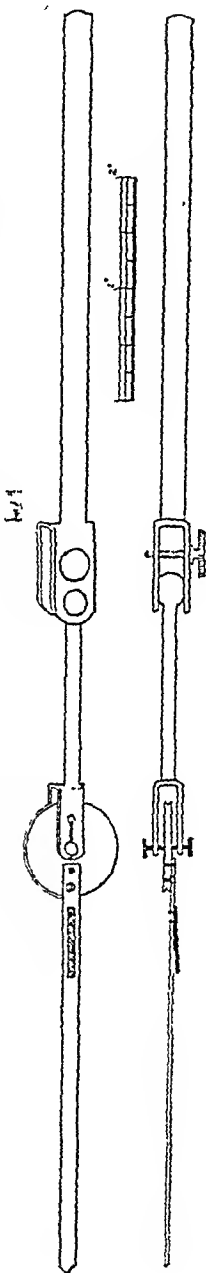


Adjustable lever and pulley for experimental work. By WALTER J. DILLING.

The instrument shown in the scale drawing has proved very useful for student's purposes and has withstood serious damage. The essential feature is the division of the brass rod support into two parts by a friction-tight ball and socket joint. This renders possible the rapid and fine adjustment of the wheel in any direction and saves shifting of the boss-head. The grooved wheel and lever arm are made of aluminium—somewhat stouter than is drawn. The lever arm is fixed to the side of the wheel by a pin with socket and a detachable screw—or it may be run into a tight groove—so that the arm may be removed and the wheel used as a guide pulley.

The provision of a small strip of steel (watch) spring rivetted to the lever arm enables the thread passed over the wheel to be caught quickly and firmly in the angle between the spring and the lever arm; when the length of thread is finally adjusted, a half clove hitch can be made in the angle leaving a loop from which weights can be hung and the loop made secure by a second hitch. The point of the lever arm may be modified for vertical writing, if desired.

The lever is particularly useful for work with surviving mammalian tissues, since adjustment of the vertical position of the tracing can be made from the ball and socket joint. When used as a guide pulley the practicability of placing the wheel at any angle and in any position has advantages. The cost of such a lever should not exceed 30s.



Pituitary extract and fatty infiltration of the liver. (*Preliminary Communication.*) By R. COOPE and E. N. CHAMBERLAIN.

In a series of 10 rabbits, fed on a diet of oats, bran and green vegetables, subcutaneous injection of 3 or 4 c.c. of commercial extract of posterior lobe of pituitary gland resulted in definite infiltration of the liver with fat.

We have not yet ascertained precisely how soon the increase begins, nor when it passes off—in the three cases where the animal was killed 15 to 18 hours after the injection, the total fatty acid in the liver was 8.35, 7.25 and 6.49 grams p.c. of the fresh tissue, as compared with an average of 2.97 p.c. for 7 controls.

The work is being continued and extended to other mammals.

The CO-dissociation curve of haemochromogen.

By M. L. ANSON and A. E. MIRSKY.

We have determined the carbon monoxide dissociation curve of haemochromogen prepared by alkaline hydrolysis of ox blood diluted twenty times. None of the impurities were removed. The curve can be expressed by the mass law equation

$$K = \frac{[\text{He}] [\text{CO}]}{[\text{He CO}]}$$

The value of K at body temperature, in very alkaline solution is 0.0105 ± 0.0004 when the tension is measured in p.c. of an atmosphere.

The percentage saturation was determined by analyses of the gas phase before and after saturation.

We are studying the influence of various factors on this reaction.

The acid base equilibrium in muscle. By S. ANDREWS, FLORENCE BEATTIE and T. H. MILROY.

The constituents of muscle which carry base in a form which can be secured by acids produced within the fibres are bicarbonates, alkaline protein salts and phosphates. As regards the bicarbonates the concentration is a very low one, even in the absolutely fresh muscle (frog) not amounting to more than 0.005 molar (Meyerhof).

In the expressed juice of mammalian muscle the only base-carrying systems of importance are the phosphates and the proteins.

As regards the phosphates the concentration in the freshly-expressed cooled juice is much higher than the bicarbonate, varying in different

pelvic end. The tension lever used had a magnification of 15.5 times, and was photographed simultaneously with the galvanometer string. Stimulation was by break induction shocks, varied in intensity by altering a resistance in the primary of the coil.

The results show that the electrical disturbance and the tension developed increase in parallel manner as the stimulus is made stronger. This is shown in the figure in which the electrical response is plotted as p.c. of the maximum P.D. developed and the tension in grams also as a p.c. of the maximum. It will be seen that the ratio of the size of the electric response to the grams tension developed remains practically constant. According to the work of Lucas(1) and Pratt(2), the strength of the contraction increases, within limits, with the stimulus because the number of fibres in action increases and not because of any change in the response of each fibre. This suggests that variations in the magnitude of the electric response of the muscle are due to similar causes. This view is supported by Gotch's(3) work on the time relations of the response in nerve and by the observations of Adrian(4) on muscle stimulated with a Pratt electrode.

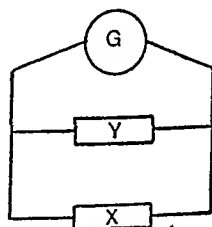
If this view is correct, we must suppose that when only a few fibres are excited the inactive fibres act as a short-circuiting path for some of the current from the active fibres. When all, or nearly all, the fibres in the muscle are contracting, however, the number of short-circuiting fibres is so reduced that the resistance of this path for the current is greatly increased and more current flows through the galvanometer. By assuming that the resistance of an active and an inactive fibre are approximately equal, it is possible to calculate the current that flows through the galvanometer when different numbers of fibres are excited.

Let G = conductivity of the galvanometer.

„ X = „ „ active fibres.

„ Y = „ „ inactive fibres.

„ e = E.M.F. produced by each fibre.



The conductivity of the whole circuit is:—

$$\frac{1}{\frac{1}{X} + \frac{1}{G+Y}} = \frac{X(G+Y)}{G+X+Y}.$$

The current through the galvanometer =

$$\frac{e \cdot X(G+Y)}{G+X+Y} \cdot \frac{G}{G+Y} = \frac{G}{G+N} \cdot e \cdot X,$$

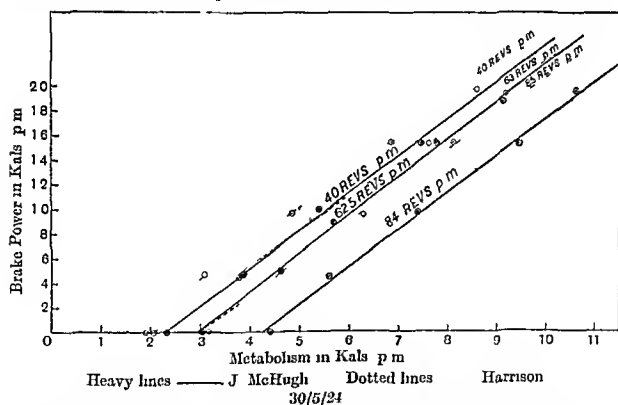
when $X + Y = N$ Thus the current should be directly proportional to the number of active fibres. If the mechanical tension developed is also proportional to the number of active fibres the ratio of the size of the electric response to the grams tension developed ought to remain constant, as it is found to do experimentally

- (1) Keith Lucas This Journ 33, p 125 1905
- (2) Pratt. Amer Journ Physiol 43, p 159, and 44, p 517 1917
- (3) Gotch This Journ 28, p 395 1902
- (4) Adrian Arch Neerlandaises, 7, p 330 1922

Relation between speed and efficiency (*continued*)

By F A DUFFIELD and J S MACDONALD

Continuing the work previously reported(1), another subject, "J McHugh" (age 20, weight 79.5 kilos, height 6 ft), has been examined cycling at different rates and with various values of the brake. The "brake" range being from 0 to 1.9 kals per minute, that is to say, from 0 to about 0.18 "horse power," and the "revolution rate" from 10 to



81 per minute. The results are represented by the heavy lines in the accompanying chart. Attention is drawn to the fact that these lines are straight, are almost parallel(2), but that they are not equidistant as in the case of Harrison. These results therefore cannot be expressed so simply as in that case, where the statement $Q = \theta K + \phi V$ was obviously admissible. Some more complex statement must therefore be found to unite the two cases.

On the same chart in "dotted lines" the previous results with "Harrison" are plotted out so as to render comparison possible. The main difference is the very marked displacement of the heavier subject's lines along the abscissa. There is a marked payment for increase in weight. The "cost of movement" that is the distance from the origin to the feet of the sloping lines varies with $W_0^{1.44}$ of the subject's "stripped weight," and with a still greater power of the subject's unmodified weight(3). There is therefore no possibility whatever of stating the "cost of movement" as equal to $xW_0^{\frac{2}{3}} + yIVV^2$ as two fractions representing the basal metabolism and a cost for "velocity."

There is, however, also another very important distinction between the two cases. "Harrison's" lines (dotted lines) make a definite angle with "McHugh's" lines (heavy lines). Now with regard to the slope of these lines it is in the first place clear that if they were vertical then there would be no "cost of work," and that the more they slope the less this is true, therefore the more erect lines of the heavier subject show definitely a greater efficiency associated with a greater weight. The variation as in the direct calorimetric experiments is such that the "efficiency" varies directly with $W_0^{\frac{2}{3}}$. With regard to the meaning of this variation which might be read as either the "disefficiency coefficient"

$$E = \frac{\text{a constant}}{\text{surface}} \text{ or possibly as } \frac{\text{a constant}}{\text{weight} \times \text{height}}.$$

Equations have been made with the data published in 1916(2) in the form $\log E = x + y \log W_0 + z \log H$ so as to discuss this meaning and their result is as follows:

$$E = \frac{KH}{\text{surface} \times W^{\frac{1}{3}}}.$$

So that the "efficiency," which is the reciprocal of E varies with the surface of the body, and with the ratio between the cube root of the mass of the body (muscularity) and the height.

Returning to the cost of movement and the mentioned impossibility of stating it either as ϕV , or as $xW_0^{\frac{2}{3}} + yWV^2$, attempts have been made to express the two sets of results in various ways, the best of which seems to be the following:

$$\text{The total cost} = \frac{xW_0^{\frac{2}{3}}}{1 + \frac{zV}{\phi(W_0)}} + yIVV^2 + EK.$$

The first of these three fractions represents the "basal metabolism" as

being diminished with increasing rate of movement, which indeed is very probably the case, and it, at the same time, serves to express the great variation, already alluded to, in the "cost of movement" of subjects of different weights.

REFERENCES.

- (1) F. A. Duffield and J. G. Macdonald. Proc. Phys. Soc. 58. Dec. 15, 1923.
- (2) Capt. M. Greenwood. Proc. Roy. Soc. B 90. 207. 1918.
- (3) J. S. Macdonald. Proc. Roy. Soc. B. 89. 403. 1916

The relation between "basal metabolism" and "cost of movement." By J. S. MACDONALD.

The total cost of cycling, given in "Kals" per minute, of the two subjects now very completely examined is expressible, as follows, in three fractions (W_0 is the weight of the unclothed subject)

$$Q = \frac{0.138 W_0^{\frac{2}{3}}}{1 + \frac{1}{W_0^{3.116}}} + \frac{5.771 V V^2}{10^6} + \frac{10^{1.733}}{W_0^{\frac{2}{3}}} K.$$

Added together, the two first fractions represent the cost of movement; the last fraction represents the cost of work. In the last fraction K is the load, or, "brake power" in "Kals" per minute and the figure by which it is multiplied is the constant "disefficiency coefficient," which varies *inversely* with $W^{\frac{2}{3}}$, and therefore whether essentially, or for some secondary reason, inversely with the surface of the body. *The greater the surface the less the cost of work.*

The first fraction is notable as having an existence when there is neither external work " K ," nor movement " V ," since when " V ," the revolution rate per minute, is naught, the fraction becomes $0.138 W_0^{\frac{2}{3}}$, and may then be styled the "basal metabolism" for the cycling posture, that is " B ." Its value then is larger than the "resting metabolism," since equivalent, in a subject of ordinary weight, to 67 kals per hour per square meter of surface.

When movement begins, this fraction is reduced since the denominator then enlarges. The reduction is represented as a very substantial one, the fraction being halved at 45 revs. per minute, and brought down to a third of its value at 90 revs. per minute. Now this denominator, expressed in "c.g.s." units may be shown in the following two forms:

$$(1) \quad 1 + \left\{ \frac{E e^{12}}{(2\pi)^5 B} \right\}^{2.337} 10^{-.013 n},$$

in which E and B have the same meaning as above, $\frac{e^{12}}{(2\pi)^5}$ represents a number of calories, and 10^{-013} seconds, as a time factor, eliminates the influence of " n ," the number of "strides per second" (double the number of revs. per second). Treated in this way the denominator represents a "pure number," which is of importance.

$$(2) \quad 1 + 2\pi \left\{ \frac{2(\pi\theta)^3}{w_0} \right\}^{3.116} 10^{-017} n,$$

in which " θ " is a constant of the value $10^{.9658}$, or $\sqrt{10\pi e}$; and the exponent 3.116 may be written in the form $\frac{2}{3}(1 + \log_e 4\pi^2)$. This second mode of expression can only be considered as that of a pure number if $(\pi\theta)^3$ can be referred to as a "mass."

Now as to the real significance of this constant " θ " some very definite connection with thermal "quantities" may be inferred from the fact that the "mechanical equivalence" of heat may be stated thus:

$$1 \text{ calorie} = 10^{7.622} \text{ ergs} = 2\pi \left(\frac{2\pi^2}{e^3} \theta \right)^4 g \text{ ergs.}$$

If in this expression $\frac{2\pi^2}{e^3} \theta$, i.e. $.981\theta$, may be taken as a "length," and its cube as a mass, the calorie is arranged as if work was done in lifting a mass, through a distance, against gravity. The distance, $.981\theta$, is interesting because as a matter of fact $\theta = \frac{L_s T_s}{L_0 T_0}$, where L_s and L_0 are the latent heats of ice and water, and T_0 and T_s , the freezing and boiling points on the "absolute scale," namely, 273° and 373° ; and thus the magnitude of the path is proportional to the ratio between the two extreme states of water, or to a "power" of that ratio. Taking the $\frac{e^{12}}{2\pi^5}$ calories from the first of these forms

$$\frac{e^{12}}{2\pi^5} \text{ calories} = \frac{e^{12}}{2\pi^5} \times 2\pi \left(\frac{2\pi^2 \theta}{e^3} \right)^4 g \text{ ergs} = (\pi\theta)^4 g \text{ ergs,}$$

a fact which shows the relation between the two forms.

Two other points might be alluded to with regard to the value of " θ ." Thus $L_s = 2\pi\theta^2$: and again if the time taken by one calorie in traversing one cubic centimeter along a temperature gradient of one degree = t , then $\pi^2 t = \theta^4$. A further point is to distinguish this "length" from the interesting "time" $10^{.968}$ seconds, during which a body would fall to such

a distance as would require a "calorio" of work to replace it, this "time," " θ_2 ," is equal to $\frac{t}{8\pi^2}$.

In conclusion attention is drawn to the need for inserting a "dis-efficiency coefficient," such as E_1 in the first two fractions as well as in the last one. Such an insertion would convert $xW_0^{\frac{2}{3}}$ into E_1W_0H , and in the second fraction zWV^2 , into $E_2xWV^2 \times W^{\frac{2}{3}}$ as is probably required by the fact that the main resistance to movement is the "wind resistance."

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

July 5, 1924.

The method of transport of oxidised carbon from the tissues to the blood. By B. J. COLLINGWOOD.

The question whether H_2CO_3 is a strong or a weak acid is of some physiological importance; and the facts which have been demonstrated in attempts at its elucidation throw considerable light on certain vital processes. The belief that it is a strong acid has gained ground in recent times. Thiel and Strohecker (*Berichte d. d. chem. Ges.* 47, p. 945. 1914) have produced evidence in favour of this view. This evidence tends to show that in a 0.00812 molar solution of CO_2 in water at $4^\circ C$. only 0.67 % is present in the form of the acid, the remainder being in solution as a gas. They found that, on the above supposition, the fraction of the acid dissociated was 0.91. The gist of their evidence was based on the delay that occurred in the neutralisation of an equivalent amount of alkali by this solution of CO_2 , and on the disappearance of this delay when the alkali solution was of a much greater dilution.

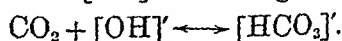
A similar delay can be demonstrated by the two following methods:

(1) If CO_2 be bubbled for two or three seconds through a weak alkaline solution containing an indicator (phenol red) and precautions taken to remove CO_2 from the surface of the liquid immediately the bubbling has ceased, a delay occurs (in certain experiments as long as 30 seconds in duration) before the solution shows an acid reaction.

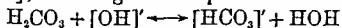
(2) If a weak alkaline solution containing the same indicator be run down a slightly inclined tube and exposed for a certain length of the tube to a mixture of CO_2 and air, the colour change does not occur until the fluid has passed a considerable distance beyond the CO_2 area. The ' CO_2 area must, of course, be strictly circumscribed; and this is brought about by a stream of air being drawn up through that portion of the tube which lies beyond the CO_2 area.

Such experiments can be explained on two hypotheses, namely:

(1) H_2CO_3 (whether ionised or unionised) is present in small amounts, and the delay is due to the time occupied in the further formation of $[HCO_3]'$ under the action of $[OH]'$, according to the equation



(2) H_2CO_3 is present in large amounts, but it is very slightly ionised, and the delay is due to the time occupied in its further ionisation under the action of $[\text{OH}]'$, according to the equation



The second hypothesis appears improbable on the grounds that ionic equilibria are very rapidly established

Whatever the cause of the delay may be, the fact of its existence shows that the passage of oxidised carbon in the form of CO_2 from the tissue fluids to the blood is an unsatisfactory method. If the alkali of the blood and of the red corpuscles is to be of any real value in expediting this transit, it is essential that the reaction of the CO_2 with this alkali should be a rapid reaction and not, as the above experiments demonstrate, a slow reaction. The time occupied by the blood in passing through the capillaries is so short that a delayed reaction would be robbed of its utility as a means of lowering the CO_2 tension in the capillary blood and thus aiding its transit. Furthermore the fact that approximately 19/20 of the CO_2 is held in the tissue fluids and blood in the form of $[\text{HCO}_3]'$ and $[\text{CO}_3]''$ renders it still more probable that CO_2 in gaseous solution is not the main means of transit of oxidised carbon from the tissue fluids to the blood.

The exchange between these two fluids would, therefore, appear to be largely one of ions and only to a small extent of dissolved CO_2 , $[\text{HCO}_3]'$, for instance, passing from the tissue fluids into the blood, whilst $[\text{OH}]'$ and $[\text{CO}_3]''$ pass from the blood into the tissue fluids. It should be noted that to maintain electrical equilibrium it is necessary that for every ion of $[\text{CO}_3]''$ that passes two ions of $[\text{HCO}_3]'$ must pass in the opposite direction. It is not suggested that a full account is given here of ionic movement, for the matter is complicated and cannot be dealt with in a preliminary communication.

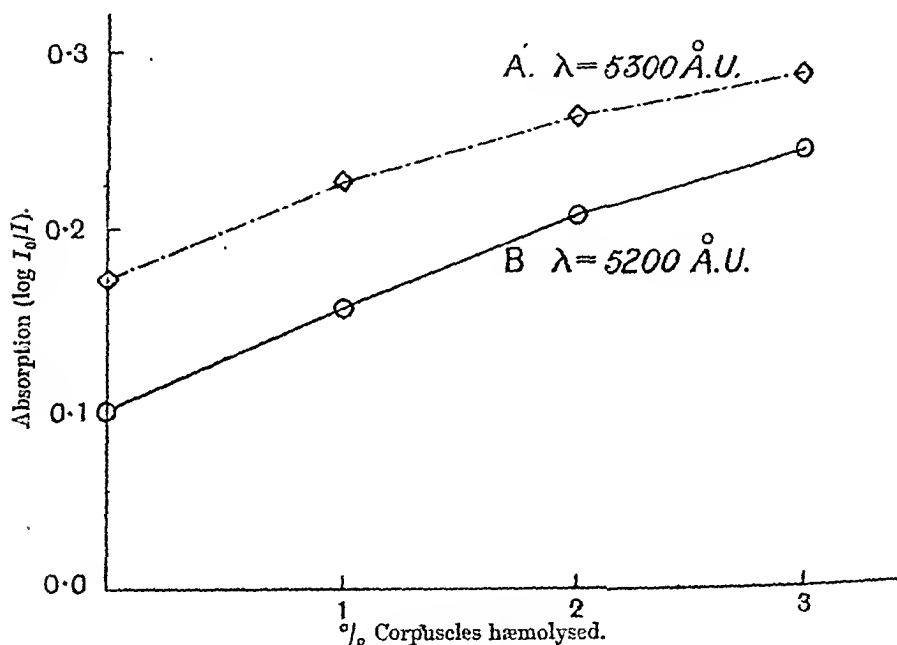
The detection of very small degrees of hæmolysis.

By J A HEWITT, F OLDHAM and C S WHITE

Methods at present in use for determining the degree of hæmolysis become unreliable when only a small proportion of the corpuscles is broken down. For example, it is impossible to differentiate with accuracy between two specimens of blood which contained originally the same number of erythrocytes, but which have, say, 1 and 2 or 2 and 3 p.c. of their corpuscles respectively hæmolysed. It has been found however that such small differences can be recognised with certainty by determining the absorption of the blood photometrically.

The Nutting Photometer suggested itself for this purpose and has been employed in conjunction with the Constant Deviation Spectrometer. Both the instruments referred to were by Messrs A. Hilger & Co. After some few experiments the most suitable regions of the visible spectrum for observations were found to be those corresponding with $\lambda = 5200 \text{ \AA.U.}$, and 5300 \AA.U. , and in the graphs appended are two records obtained in the same specimens of blood in which these sources of monochromatic light were used.

The two solutions *A* and *B* were made up to contain the same total amount of blood, but were so adjusted as to have varying percentages



of the corpuscles hæmolyzed. In the observations given these percentages varied from 0 to 3. The absorption is calculated from the values $\log I_0/I$, where I_0/I is the ratio of the intensities of the light entering the medium to that transmitted by it.

Regarding the applicability of this method it might be well to indicate that while one of precision, it does not readily lend itself to rapid observation, and it does not seem possible at present by its means to follow the actual progress of hæmolysis unless this process were very slow. On the other hand, for comparison of transparent solutions such as hæmoglobin or other pigments, the instrument will yield in all probability results more accurate than those obtained by the methods in common use.

It is proposed to continue these observations.

The effect of irradiation with the carbon arc on pigs on a diet high in phosphorus and low in calcium. (*Preliminary Communication*) By J B ORR, H E MAGEE and J M HENDERSON

A considerable amount of work has been done on the effect of ultra violet light on animals fed on rickets producing diets, but, while growth curves have been studied and clinical observations made, very little has been done from the point of view of following quantitatively the changes in the calcium and phosphorus metabolism

The present investigation was undertaken with this object. Two pigs, comparable as regards sex, age and weight were kept in semi-darkness in metabolic cages. A ration was fed which consisted of

Oatmeal	180 gms	per day
Maize	270	" "
Middlings	450	" "
CaCO ₃	6	" "
Distilled water	Ad lib	" "

The intake per 48 hours was accordingly 8 230 gm CaO, 20 366 gm P₂O₅ and 36 072 gm N. A comparison of the mineral ratios of this diet and of sow's milk shows the diet to be ill balanced with regard to inorganic constituents. Thus

		Sow's milk	Diet
CaO	P ₂ O ₅	10 9	10 25
CaO	MgO	20 1	20 19
Na ₂ O	K ₂ O	5 2	5 14

Urine and faeces were collected separately over 48 hour periods and the calcium, phosphorus and nitrogen balances determined.

In the 10 days pre irradiation period the retention of calcium and phosphorus in both animals showed a decrease which was most marked in the case of calcium. In the control animal, which was not subjected to irradiation, this decrease continued and loss of appetite and gastrointestinal disturbances supervened.

Following the 10 days pre period the other animal was subjected to irradiation for a period of 24 days. The irradiation consisted of one hour's exposure daily to a carbon arc lamp at a distance of three feet.

The chief results are as follows:

(1) A definite increase in the calcium and phosphorus absorption and retention in the experimental animal, the balance curves for these minerals approaching each other in the light period and running a parallel course.

(2) The excretion of calcium and phosphorus in the urine rises both actually, and relatively to their excretion in the faeces. There is, therefore, either an increased absorption from the gut, or a decreased re excretion.

(3) These results take place in the experimental animal without any concomitant increase in body weight over a period of about six weeks. Thus the weight at the beginning of the experiment was 21.5 kilos, and 22.4 kilos at the end. This would indicate that a stimulus to calcium and phosphorus retention does not necessarily involve a stimulus to growth in general.

The following table gives the composition of the bones of the irradiated pig and the control pig.

BONE	EXPERIMENTAL PIG		CONTROL PIG	
	Tibia and fibula	Radius and ulna	Tibia and fibula	Radius and ulna
Wet weight	140.99		108.93	
% ash in dry fat-extracted bone ...	45.86	43.39	39.77	41.23
% CaO in dry fat-extracted bone ...	24.25	23.35	20.84	22.17
% P ₂ O ₅ in dry fat-extracted bone ...	20.32	18.39	18.12	17.39

The only abnormality observed at autopsy was an enlarged thyroid in the case of the experimental animal. The iodine content of the thyroid was very low in both cases—particularly in that of the irradiated pig. (.0193 and .0025 % respectively.)

The results of other experiments show that the effect of irradiation is less marked on a diet better balanced with regard to its mineral content. An account of this investigation will be published shortly by one of us—(J. M. H.).

A note on the calcium content of gouty sera.

By VINCENT COATES and P. C. RAIMENT.

Working with the serum of blood obtained from patients suffering from gout we have found a high content of calcium. The cases have been examined both in the acute and in the interval stage, and in each case are considerably above the normal. The following table gives the figures:

Exp.	Age	Ca in mgms. %	Remarks
1.	46	16.4	Interval.
2.	48	22.6	"
3.	52	16.4	Acute.
4.	53	20.0	Interval.
5.	63	18.4	"
6.	48	16.8	Acute.
		24.0	Interval.
7.	51	16.0	"

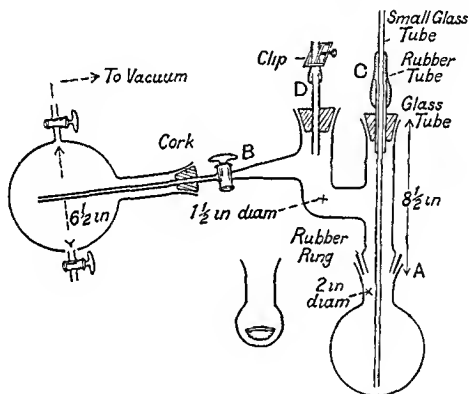
The method of estimation was that described by Kramer and Tisdall.¹

¹ *J. Biol. Chem.* 47. 475.

A modified Claisen vacuum distillation apparatus.By **RUDOLPH A. PETERS**

The apparatus¹ depicted in the figure possesses certain advantages when handling small amounts of material in large amounts of fluid

It embodies two main points (1) At *A* flasks of different cubic capacity can be fitted rapidly by rubber Gooch crucible cones. Distillation can therefore be commenced with a large flask, and the contents transferred quickly to a smaller flask, even finishing in a small glass basin as depicted. Adjustment of the capillary tube is performed as



shown at *C*. If desired a series of flasks can be ground to fit the joint at *A*. (2) *B* is a large bore tap which can be conveniently joined to the apparatus. By closing this tap before disconnecting at *A*, the vacuum in the receiver can be maintained. This saves time in recommencing the distillation.

An ordinary 5 litre distilling flask can be used as receiver, or better a special receiver shown in the figure fitted with two taps, 1, for connection to the vacuum pump, and 2, for removal of the distillate. *D* can be used for the introduction of fluid.

¹ This apparatus can be obtained from Messrs Plowden and Thompson, Dial works, Stourbridge.

The neon tube as a stimulating apparatus.

By I. DE BURGH DALY.

It has been shown by Pearson and Anson⁽¹⁾ that if a high resistance is connected in series with a neon tube having a capacity shunted across its electrodes, the continuity of the current through the tube is interrupted and it glows at regular intervals of time. A critical voltage of approximately 170 is necessary to make the tube glow.

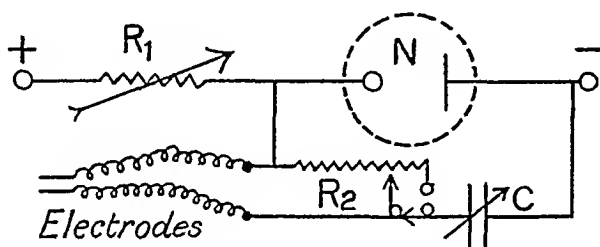


Fig. 1.



Fig. 2.

The circuit shown in Fig. 1 was connected up; it consisted of R_1 , a Watmel variable grid leak (0.5–5.0 megohms); R_2 , a variable resistance (0–4000 ohms); C , a variable condenser (0–10 μ F.) and N , an Osglim lamp with the resistance in the cap removed. The 200 volt D.C. supply in the building or dry cells were used. Adjustments of R_1 and C controlled the frequency of lamp discharge and the value of R_2 determined the strength of current used.

This arrangement is a convenient form of stimulating apparatus in which the frequency and strength of the stimulus can be rapidly adjusted to any desired values. The frequency of discharge (neglecting R_2) is given by the formula

$$T = CR_1 \log_e \frac{V - b}{V - a},$$

where

 T = duration of one dark period, C = capacity of condenser, V = applied voltage,

“ a ” and “ b ” represent the upper and lower critical values respectively of the voltage.

The duration of one dark period differs from the time of one complete cycle by less than 1 p.c.

The shape of the discharge is depicted in Fig. 2; the make shock is greater than the break. The maximum frequency of discharge is approximately 95,000 per second.

With $R_1 = .5$ megohm, $V = 180$ volts and $C \approx .1 \mu\text{F.}$, the frequency was 15 per second. Under these conditions the value of R_2 for a threshold stimulus of the frog's sciatic nerve was 185 ohms. With $R_2 = 400$ ohms the stimulus was uncomfortable to the tongue.

Increase in the frequency of discharge caused by diminution of C decreases the strength of each individual stimulus, the value of R_2 will therefore have to be adjusted in order to obtain the same threshold stimulus at different frequencies.

If one lead of the supply mains is earthed difficulty may be encountered in keeping up the interrupted discharge. Efficient insulation or the use of dry batteries will overcome this trouble.

REFERENCE.

(1) Pearson and Anson. *Proc. of the Physical Soc. of London*, 34. Part v, p. 204. Aug. 15, 1922.

A simple device whereby some colour blind (hypochromatic vision) persons can recognise colour differences. By H. E. ROAF.

Some persons with hypochromatic vision are sensitive to as great an extent of the spectrum as normal people. On testing them for their sensitivity to light I have found that they seem to have as low a threshold for light of different wave lengths as a normal person therefore their defect is in the recognition of difference between one part of the spectrum and another. By means of a colour filter it is possible to show them that the colours which have appeared to them to match are really different. For instance by means of a red screen a green and a yellow which appeared the same to one person look dark and light respectively; therefore by knowing that the screen is red the person with hypochromatic vision can decide which is yellow and which is green.

We do not yet know the right intensity of screen or shade of colour which gives the best result. This is a matter which can be decided only by experience with different individuals and it will take time to accumulate data and to learn the best way to evaluate them. It may be possible for a person to be trained to recognise colours in a normal way by means of a red tinted monocle. For persons with greater defects of colour vision than those that I have studied more than one colour screen might be necessary.

The occurrence and concentration of arginase in the organs of fishes and other animals. By ANDREW HUNTER and JAMES A. DAUPHINEE.

Using the quantitative method outlined in the preceding communication we have studied, more or less exhaustively, the distribution of arginase in seventeen species of fish and in several representatives of other orders. The following are the main conclusions reached.

1. Arginase is present in the livers of all fishes in quantities which within a single order, or even within a single family, may differ very greatly, but which for any one genus appear to be characteristic and relatively constant.

2. Of the fish livers examined the richest in arginase was that of the dog-fish (*Squalus sucklii*), the only elasmobranch in the series. It yielded from two to forty times as much as the livers of teleosts.

3. The livers of mammals, especially of carnivores, are much more active than those of teleostean fishes. The livers of Chelonian reptiles contain a little arginase, those of birds none.

4. In the herring and the dog-fish, and perhaps in other fishes also, the organ next in activity to the liver is the heart. In other vertebrates the heart is inactive.

5. The kidneys also of fishes invariably contain arginase, but in a concentration inferior to that shown by the liver. The mammalian kidney contains even less, relatively to the liver much less, than the fish's. The bird's kidney on the other hand yields more than any kidney yet encountered save the dog-fish's.

6. Among the remaining organs of fishes the distribution of the enzyme appears to be variable. It was absent from the intestinal mucosa, pyloric caecae, spleen, testis and muscle of the ling-cod (*Ophiodon elongatus*); but present in the muscle tissue of the herring, and in all organs of the dog-fish except the brain and the blood.

7. In mammals arginase could not be demonstrated in any organs save the liver and kidney.

8. The results as a whole confirm Clementi's doctrine that arginase is present in the livers of all animals which have a "ureotelic" nitrogen metabolism, and absent from the livers of those whose metabolism is "uricotelic." They afford also some basis for the further hypothesis, tentatively suggested, that the restriction of the enzyme in higher vertebrates to one or at most two organs is a condition evolved from a primitive state, still persistent in the dog-fish, in which arginase is rather generally distributed throughout the body.

9. While arginase is lacking from the ovum of the dog-fish, it is present towards the end of development in at least the muscles and the liver of the foetus; but its concentration in the foetal liver is still far below that in the adult organ.

10. Arginase is rarely, if ever, to be found in the tissues of invertebrates.

The assessment of the physical fitness of schoolboys.

By J. GILBERT WOOLHAM.

This investigation was undertaken to ascertain the character of the distribution curve of results based on an assessment of physical fitness by the Heald Thomson combination of Dreyer and Flack tests, (a) on a random sample of schoolboys, (b) on schoolboys referred for examination on account of ailments.

The Heald Thomson formula for the "Dreyer products" was modified, standard weight being calculated from the sitting height and chest girth instead of from the sitting height alone. The formula for the "Flack product" was modified (1) as regards the denominator,

$$1000 \times \frac{(\text{Age in years})^{1.807}}{4}$$

being substituted for 52,000, (2) and for the "persistence test" half the height of the Hg column in the expiratory pressure test, as found for each case, was used.

The results entered on the Heald Thomson graph gave the following:

Areas	0	1	2	3	4	5	6	7 = Scale of assessment
Boys	0	1	8	18	29	18	5	2 Distribution found
Boys	0	1	5	19	31	19	5	1 Dittq calculated on a probability basis
Boys .	1	6	7	10	7	3	1	0 Distribution of ailing boys in areas of graph

The close approximation between the series found and that calculated supports the view that this combined method of assessment is of value when applied to schoolboys. The distribution of ailing children is skewed towards the lower values; this assessment is still in progress.

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- Heald and Thomson. "The Value and Interpretation of some Physical Measurements." *Lancet*, Oct. 9, 1920.
 Woolham. "The Assessment of Physical Fitness." Report of the School Medical Officer, Manchester, Year 1923.

The action of nitrous acid upon the antineuritic properties of yeast extracts. By RUDOLPH A. PETERS.

McCollum and Simmonds¹ have shown that the *growth* promoting properties of extract of wheat embryo are but little affected by passing nitrous acid gas through the extract for four hours. I have found that the *curative* properties of yeast concentrates for pigeons suffering from symptoms of head retraction are not altered by treatment with hydrochloric acid and sodium nitrite. Treatment in different experiments was slightly varied, but consisted in the main in a treatment at room temperature or below with nitrite for several hours followed by warming and subsequent boiling before administration. The resistance to nitrous acid would seem to exclude all primary amines and a large number of secondary amines from being involved in the curative properties of these extracts.

A nitroprusside reaction given by skin. By E. WALKER.
(*Preliminary communication.*)

The nitroprusside reaction given by skin is best demonstrated by allowing relatively thick (0.5 mm.) sections to stand for a few minutes in a saturated solution of ammonium sulphate, to which is added 3-4 drops of a freshly-prepared 5% solution of sodium nitroprusside (*vide* Hopkins⁽¹⁾). A few drops of strong ammonia are then added, whereupon a magenta-coloured band develops along the epidermal edge. The reaction is a difficult one to observe with any great histological accuracy on account of its transience and it has not been possible so far to fix it in any way. But it is quite clear that the reaction is definitely located in the living layers of the skin; viz. all the layers of the epidermis with the exception of the stratum corneum. In addition, the hair-follicles and sebaceous glands are involved, whilst slight patches scattered through the dermis suggest that the sweat-glands also give the reaction. The dermis itself and the subcutaneous fat are devoid of the reaction. It is to be found also in the nails, becoming more diffuse and less distinct progressively with the length of the nail, disappearing finally towards the tip. That the reaction is a very real one and not due to any degenerative changes is readily established by its demonstration in the living subject. If the finger-nail be freshly cut, flush with the finger-tip, and the tip of the finger then immersed in the necessary reagents for 5-10 minutes, the

¹ McCollum and Simmonds, *J. Biol. Chem.* 33. 55. 1918.

reaction can be seen quite distinctly, with the naked eye, in the form of a thin magenta line along the bed of the nail.

The identity of the compound responsible for this reaction still remains to be investigated. There can be little doubt that the reaction demonstrated is due to the presence of some sulphydryl compound, although it is a point not yet finally proven. If such is the case, it affords interesting confirmation of the generally-accepted view that the $-SH$ group is to be found associated with metabolically-active cells. This skin constituent is characterised by considerable stability and closely resembles the thermostable sulphydryl constituent of muscle described by Dixon and Hopkins(2). Certain experimental evidence, however, suggests it is not identical with it. Its full significance in connection with the skin remains to be determined. The evidence so far obtained shows that it is affected by certain skin-irritants, at least *in vitro*.

Many types of skin have been examined, human, calf, cat, pig and rabbit, and all give a similar reaction.

(I am grateful to the Staff of the Radcliffe Infirmary for their kind cooperation.)

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- (1) Hopkins, F. G. *Bioch. Journ.* 15. 288. 1921.
- (2) Dixon and Hopkins. *Journ. Biol. Chem.* 54. 526. 1922.

Allelocatalysis and the growth of yeast. Preliminary communication. By G. L. PESKETT.

The growth of brewer's yeast at 25° C. has been studied in hanging-drop cultures containing one, two and in a few cases, three cells, both in synthetic and in bios-containing media. After growth the cultures were dried and stained for bacteriological examination.

Out of 128 growing cultures 89 were free from contamination while the remaining 39 contained a few isolated bacteria, so few in comparison with infected controls as to suggest contamination during the drying process.

No evidence of allelocatalysis was obtained save in the case of three cultures out of the 128 studied, although the conditions in the bios-containing media were analogous to those under which T. B. Robertson¹ has demonstrated the occurrence of this phenomenon in the case of *Enchelys*.

¹ *Biochem. J.* 15. 612. 1921.

An indicator method for the estimation of urea and its application to the determination of arginase. By ANDREW HUNTER and JAMES A. DAUPHINEE.

Urea, in concentrations up to 0.15 p.c. can be estimated fairly accurately by decomposing it with urease in the presence of 0.05 M phosphate at a *pH* of 6.8 and determining colorimetrically the resultant change in hydrogen ion concentration. The method has been utilized for the determination in an approximative way of urea in urine and in elasmobranch blood. It has been made also the basis of a convenient and reasonably exact procedure for the detection and estimation of arginase. The arginase-containing extract is allowed to act under specified conditions of temperature and hydrogen ion concentration upon a standard solution of arginine, and the urea produced in a given time is determined in the manner just indicated. The method is standardized by determining the urea produced in the same time and under the selected conditions by known relative concentrations of arginase.



PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
October 18, 1924.

Active principles of the pituitary posterior lobe.

By W. O. FENN.

A new method has been developed by means of which the effectiveness of extracts of the posterior lobe of the pituitary body, in causing expansion of the melanophores of the frog, can be judged more rapidly and with far greater sensitiveness than by the usual method of injections into the dorsal lymph sac. The frog is pithed and perfused from the bulbus arteriosus with Ringer's solution until all the melanophores are completely contracted. This involves considerable œdema, which, however, does not appear to interfere with the test. One front and one hind leg are then tied off tightly as controls and perfusion is continued with Ringer's solution containing a given amount of the extract. Within about 8 minutes a distinct darkening of the perfused leg in comparison with the control begins to appear. This colour change is complete in 20-30 minutes. By a number of trials a threshold concentration of the unknown solution can be selected, which is then taken as the equivalent of the threshold concentration of a known solution.

Fresh posterior lobe extracts have been extracted with butyl alcohol, either by the continuous extraction method used by Dudley, or merely by shaking, centrifuging and decanting two or three times. Both the extract and the residue have then been evaporated to dryness at 40° C. in vacuo, taken up in equivalent volumes of Ringer's solution and tested (a) for the oxytocic principle on the guinea-pig's uterus, (b) for the pressor principle in spinal cats, (c) for the melanophore principle as above, and, in one case, (d) for the diuretic principle in a cat under urethane. In a typical case the residue was three times as strong as the extract in the pressor and the diuretic principles, roughly 10 times as strong in the melanophore principle, but only one-sixth as strong in the oxytocic principle. This confirms the conclusion of Dreyer and Clark who found that the melanophore stimulant and the oxytocic

principle could be separated by ultrafiltration, as well as the conclusion of Dudley that the pressor and the oxytocic principles are distinct. The pressor, diuretic and melanophore principles are, however, not separable by butyl alcohol.

The test on the melanophores is one of extreme delicacy, preliminary results showing that it will detect one part of fresh gland in 10^{10} parts of solution, and one part of a commercial extract in 10 million parts of solution. Attempts were therefore made to use the test for the detection of the pituitary principle in the circulating blood. For this purpose 20 c.c. of blood is defibrinated, centrifuged, diluted until isotonic with frog's blood, diluted further with an equal volume of Ringer's solution (if necessary to increase its volume) and perfused through a washed frog. Normal human or rabbit blood gives little or no effect under these circumstances. 0.3 c.c. of commercial pituitary extract injected into the ear vein of a rabbit can, however, be detected readily in blood drawn 15–30 minutes later. No reaction was obtained from blood taken during insulin convulsions in a rabbit, nor after intravenous injection of adrenaline, nor in human blood taken during a diuresis caused by drinking large quantities of water. The test must be used with considerable caution, however, for it can be produced occasionally in other ways than by pituitary extracts, as by relatively strong (0.1 to 4 p.c.) extracts of ovary, thymus, appendix, and of commercial thyroid tablets. A marked reaction obtained once from blood drawn 15 min. after intravenous injection of ovarian extract into a rabbit, was duplicated by the addition of an equivalent amount of the same extract to normal blood *in vitro*.

On the action of histamine. By R. J. S. McDOWALL
and B. L. WORSNOP.

Large doses of histamine have been shown by Dale and Laidlaw to cause a prolonged fall of arterial pressure as a result of capillary dilatation. The condition is analogous to severe hæmorrhage. The evidence below indicates that small doses produce a condition analogous to that of a small hæmorrhage; *i.e.* the effect of small doses of histamine is not so evanescent as the very temporary fall in arterial pressure suggests. The blood-pressure recovers largely as a result of the increased peripheral resistance which is brought about. The evidence is as follows:

1. Although the blood-pressure recovers from a small dose of histamine, the animal is less capable of recovery from a subsequent dose. Eventu-

ally, if a succession of doses be given, a small dose which would be recovered from normally causes a prolonged fall of blood pressure

2 When the blood pressure has recovered, there is increased arterial tone as indicated by the increased response to acetyl choline

3 There is contraction of the aorta as indicated by the increased velocity of the pulse-wave

4 Many of the plethysmographic tracings of Dale and Richards indicate that after the immediate effect of the histamine has passed off the final volume of the limb is reduced

5 A vaso pressor reflex has been shown by McDowall to be brought into action

6 Apart from the immediate and temporary effect of the histamine a succession of doses from which the arterial pressure recovers, brings about a progressive fall of venous pressure

All the above results are given alike by small hæmorrhages and small doses of histamine, and the effect of histamine is to be considered cumulative in the same sense that the effect of hæmorrhage is cumulative Preliminary experiments show that similar constriction of arteries may be brought about if histamine be absorbed from the alimentary canal, although it is not claimed that the constriction is entirely reflex The importance of this latter observation lies in the fact that histamine is known to be a normal constituent of the intestinal content, and abnormal intestinal absorption is strongly suspected of bringing about conditions of arterial constriction in man

The effect of splenectomy on carbon monoxide poisoning.

By J BARCROFT, C D MURRAY and J SANDS

A series of papers (1)-(5) recently published has led to the view that the spleen under circumstances which call for an accession of hæmoglobin in the blood can by contracting, add to the amount of that pigment in circulation On this view the spleen would act as a reservoir or floating balance to be drawn upon in case of such emergencies as exercise, carbon monoxide poisoning, hæmorrhage, exposure to heat, etc The above papers do not give any clear indication of whether this property of the spleen is quantitatively trivial or whether it is sufficiently developed to be regarded as a definite function, *i e* whether the possession of a spleen makes any tangible difference to the animal in this connection

The experiments to be described were designed to test whether in guinea pigs exposed to the influence of carbon monoxide, those which

have been splenectomised die sooner than either normal guinea-pigs, or pigs which have had the abdomens opened, the spleen exposed and some tissues (pancreas or omentum) cut out. The operation is done four days before the exposure of the animals to coal gas.

The following table is made up from the data of six separate experiments, in each experiment the average time which elapsed between the commencement of the exposure and the deaths of the *normal* guinea-pigs is called 100 and the other times are worked out on that basis. The actual average period of longevity of the normal guinea-pigs is usually about 70 minutes.

	Normals	Operated controls	Spleneecto- mised
Number of observations	16	15	16
Mean longevity in gas	100	93.2	72.1
Standard deviation	8.14	13.02	17.86
Standard error	2.03	3.36	4.46
Percentage of COHb in blood at death	83.3	83.5	83.3

It appears that the difference of longevity between the splenectomised animals and either the normal operated controls or the normals is significant while it is doubtful whether there is any significant difference caused by operative shock between the normals and the operated controls.

Similar experiments have been carried out in which coal gas is replaced by hydrocyanic acid gas, in such the possession of a spleen should, on the above argument, make no difference to the longevity and in fact it does not do so.

- (1) Barcroft, Binger, Bock, Doggart, Forbes, Harrop, Meakins and Redfield. Phil. Trans. Roy. Soc. B. 211. p. 419.
- (2) Barcroft, Meakins, Davies, Scott and Fetter. Ibid. p. 435.
- (3) Barcroft, J. and H. Journ. Physiol. 58. p. 138. 1923.
- (4) Hanak and Harkavy. Ibid. 59. p. 121. 1924.
- (5) Carroll and de Boer. Ibid. In press.

The *v* wave in the venous pulse. By D. T. BARRY.

There is fairly general agreement among physiologists concerning the mechanism by which the *v* wave is formed in the jugular pulse, but there are a few at least who are still in doubt about it. Most people would say that the ascent of the wave is due to stasis during ventricular systole, and its decline to the entry of blood into the ventricle during diastole. The angle of junction between these two comparatively slow processes should present a rounded rather than a pointed form, and this it does

with the ordinary methods of recording. But with delicate optical recorders the highest point of the *v* wave is shown to be a sharp spike (Fig. 1), which must be due to some more active and more rapid agency than those mentioned. Some other causes or factors in the formation of *v* have been mentioned. Mackenzie(1) ascribes it at least to some extent to tricuspid regurgitation which he says is fairly common, and also alludes to the effect of push on the *a-v* valves according to the idea of Gerhardt and Wenckebach. Lewis(2) interprets the suggestion of these latter authors as a rebound from the *a-v* ring. Saintsbury, writing in one of the medical journals, remarked that even with levers the apex of *v* was sometimes too pointed to be explained in the usual way but offered no other explanation.

The spiked oscillation which makes the real summit of the *v* wave occurs immediately after the beginning of the second sound and is one

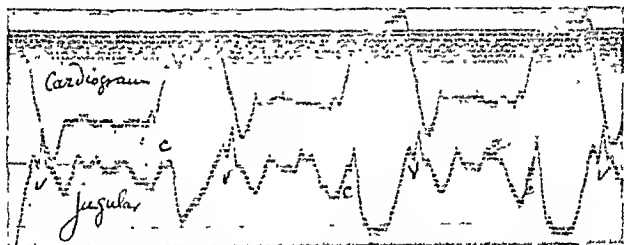


Fig. 1. Cardiogram and venous pulse. Note sharp spike on *v* wave. Time $\frac{1}{100}$ sec.

of a series of vibrations, generally not the first, on the top of the wave which correspond to the second sound. At a short interval after this spike there occurs a blunt shoulder which I think marks the point of opening of the *a-v* valves. With a sensitive sound tambour placed over the jugular vein in some subjects the effect of stasis is cut out, and instead of a *v* wave we get a series of vibrations agreeing in position and frequency with those of the second sound taken at the apex. So the vibrations of the semilunar valves undoubtedly affect the venous pulse. For some time I was inclined to adopt the view that one of these, for some reason larger than the others, caused the spike in question. But in several curves it is seen that the rhythm of the vibrations is disturbed at the point of its occurrence and it seems to be independent. Some records taken with a sound tambour to the right of the apex beat sug-

gested another agency which might explain the formation of the spike. These records resemble in some ways an inverted cardiogram and in other respects a phonogram in which the second sound is replaced by a sharp upstroke, the height of which varies somewhat with different phases of respiration. This upstroke corresponds very closely with the *v* wave in the venous pulse. It can only be due, I think, to a sudden increase of intrathoracic pressure at the outset of diastole, before the filling of the heart with blood in diastole which is a comparatively slow process of later occurrence.

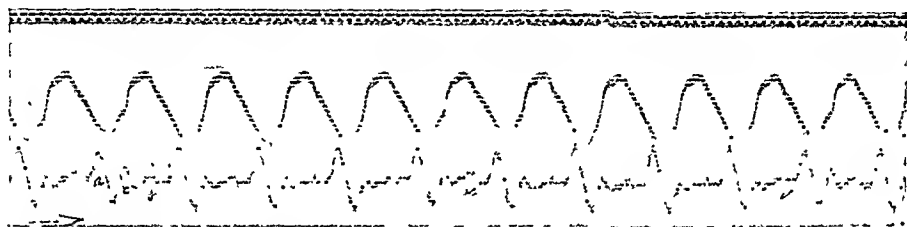


Fig. 2. Right intraventricular pressure record (top) and superior vena cava. From a dog with thorax open and innominate artery tied. A series of vibrations is seen at the position of the *v* wave in each venous cycle.

The top of the spike referred to is taken to represent the apex of the *v* wave, and it is believed that even in crude records without a spike the highest point of the *v* wave may precede that of the opening of the *a-v* valves by a distinct interval. It is of importance to locate this point exactly because, failing a phonogram, it gives the best indication of the beginning of diastole.

The absence of a *v* wave in venous records taken with the chest open, at any rate of a sharply formed wave, though semilunar vibrations may be present (Fig. 2) is in support of the view put forward.

REFERENCES.

- (1) Mackenzie. Amer. Jour. Med. 134. p. 12. 1907.
- (2) Lewis. Brit. Med. Jour. 1482. 1908.

The action of quinine and quinidine on frog's nerve.

By SYBIL COOPER.

In recent years a large amount of work has been carried out on the action of quinine derivatives on the heart. Quinidine is the most effective derivative and most workers agree that its action is due to a lengthening of the refractory period of the heart muscle. It was thought that some light might be thrown on the action of these drugs by studying their

effect on peripheral nerves, especially on the least interval for muscular summation of these nerves. Methods were used similar to those described by the writer for the action of certain acids on nerve(1); a frog's sciatic gastrocnemius was used and the nerve could be surrounded by either Ringer solution, or a solution of the drug in Ringer, it was stimulated by a fluid slot electrode. The results, which can be best shown in a tabulated form, give no reason to suppose that either of these drugs acted in a way markedly different from other narcotics.

Exp.	Temp °C.	Drug	Normal least int. secs.	Prelim. fall secs.	Time to prelim. fall mins.	Final value of least int. secs.	Time to final value h. m.
1.	18	1/1000 Quinine Sulphate	002	0018	3	0045	6 0
2.	17.5	1/100 Quinine Hydrochloride	002	0019	2	< 016	6 35
3.	17.5	1/100 " "	0017	—	—	> 0054	1 49
4.	17	1/100 Quinidine Sulphate	0023	0022	2	< 016	0 25
5.	16.5	1/250 " "	0021	0017	2	> 006	2 55
6.	17	1/250 " "	0022	002	2	02	4 0
7.	16.5	1/250 " "	0021	0018	2	0095	3 10
8.	16.5	1/500 " "	0021	0018	3	005	4 45

These results all show a preliminary fall of the least interval, then after a steady period the interval rises. During the final rise the contractions got smaller, summation was difficult to detect, and when the drugs were replaced by Ringer solution there was no recovery; these all indicate that the drug gradually kills the nerve. Weak doses either had no action, or acted very slowly. When the least interval could be measured with any degree of certainty just before conduction failed, there was absolutely no indication of a final value greater than the value for the total refractory period of the normal nerve, and therefore no reason to suppose that the refractory period or the rate of recovery were lengthened.

REFERENCE

- (1) Cooper Journ Physiol 59 p 32 1924.

The influence of hydrogen ion concentration upon conduction in the mammalian auricle¹. By A. N. DRURY and E. COWLES ANDRUS².

The dogs' hearts used in these experiments were perfused *in situ* by a modified Langendorff method, with Locke's solution whose p_H was varied from 7.8 to 7.0 by the addition of NaHCO_3 or HCl (1). The rate of conduction in the auricle was measured electrically by placing upon

¹ Observations undertaken on behalf of the Medical Research Council.

² Fellow in Medicine of the National Research Council, U.S.A.

the muscle two or more paired non-polarisable electrodes in line and connecting these in the usual way with galvanometers.

With a well oxygenated perfusate of p_H 7.4 the rate of conduction lay around 800–1000 mm. per second, both in the heart beating naturally or responding to rhythmic shocks at rates around 150 per minute; figures consistent with those observed in the intact animal. If the p_H of such a perfusion fluid was made slightly more alkaline (p_H 7.8) the rate of conduction was increased; while if it was less alkaline (p_H 7.0) it was always slowed. These changes were seen, not only in the heart driven rhythmically at rates around 150, but also in the heart beating naturally. In the latter circumstances the sinus rate was accelerated by the more alkaline (p_H 7.8) and retarded by the less alkaline (p_H 7.0) fluid so that the rate of conduction and the rate of the sinus rhythm moved hand in hand. With the more alkaline perfusate, the electrical record remained apparently unchanged, but with the less alkaline the amplitude was reduced and the rate at which the upstroke and downstroke of the intrinsic deflections was written was decreased; the extent to which the electrical record was degraded being intimately associated with the degree to which conduction was slowed.

A series of experiments was made with a perfusate of p_H 7.0 in which the oxygen was replaced by nitrogen. Under these conditions the rate of conduction was very considerably retarded. Moreover this retardation was not uniform throughout the muscle, but became progressively greater as the excitatory process moved further from the point of stimulation. For instance, if the auricle were being driven rhythmically from the base, and three pairs of non-polarisable electrodes were placed equidistantly along the body and appendix, the rate of conduction between the proximal and middle contacts was greater than that between the middle and distal contacts. On many occasions, the excitatory process failed to reach the distal contact at every rhythmic stimulation, so that a 2 : 1 response was there recorded, with a 1 : 1 response at the proximal and middle contacts. At a later stage the tissue under the distal electrode failed entirely to respond, and there appeared a block of lesser degree at the middle electrode, while the proximal electrode still recorded a response to each rhythmic stimulus. That this phenomena was not due to the tip of the appendix being more affected by the perfusate than the body of the auricle, was shown by stimulating both the body of the auricle and the tip of the appendix in the same experiment. The excitatory process was then found to be progressively slowed as it approached the tip of the appendix or the body of the

auricle, or died out before it reached the furthestmost contact. The electrical records during such a condition showed a greater degree of degradation the further they were taken from the point of stimulation. Acetyl choline in doses sufficient to produce a profound vagal effect relieved such a block.

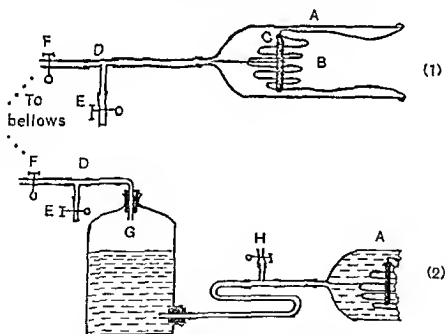
Upon returning to a well oxygenated perfusate of p_H 7.4 this progressive slowing of the excitation process was quickly abolished and the rate of conduction again approached the normal values. The similarity of this progressive slowing of the excitatory process, and dying out of the wave, to the phenomena observed in nerve and described there as "decrement" is striking.

(1) Details of the method have already been described by one of us (E.C.A.), Heart, 11. p. 97. 1924.

Simple plethysmograph for the hand for use with bellows volume recorder. By WILLIAM H. WILSON.

The plethysmograph chamber *A* in the figure is a cylindrical glass 2-litre irrigator reservoir with terminal tubulure.

A surgical rubber glove *B*, the size of which should approximately fit the subject, sizes 7½ and 8 being the most generally useful, receives the



(1) Plethysmograph chamber containing air. (2) Chamber filled with water, air-transmission to bellows recorder.

hand. To attach the glove the edge of the glass vessel is dipped in melted paraffin wax and is then allowed to cool; the cuff of the glove is slipped over the waxed edge, the hand of the glove being inside the vessel; the edge covered by the cuff is then dipped into water sufficiently hot to

melt the wax; on cooling an air-tight junction between the glass and the rubber is formed.

The plethysmograph is connected by rubber tubing through the T-piece *D* with the bellows. *F* and *E* are spring clips.

To use, the bellows connection is clipped at *F*, *E* is opened, and the air is partially exhausted from *A* by inspiration with the mouth through the rubber tube *E*. The glove is distended and the hand of the subject, previously talced, can be readily introduced (for subsequent removal, air is blown in through *E*, the subject withdrawing his hand as the glove becomes everted, the glove is drawn into place again by suction); air is allowed to return through *E* and the glove collapses on to the hand. *F* is now opened and before closing *E* sufficient air is blown in to slightly raise the writing point of the bellows (weighted sufficiently to maintain a pressure of 1 cm. of water within the apparatus).

C is a wooden handle attached to a wire fixed in the tubulure; it is lightly grasped by the hand.

For large changes of volume, *e.g.* venous distension, a bellows with a maximum volume of 25 to 30 c.c. is used, for smaller changes one of 15 c.c. A gauge is made for measurement of the curves by recording the movement of the writing point when successive volumes of 1 c.c. are introduced from a 20 c.c. syringe; the record is varnished and preserved for use.

For some purposes, as for example the demonstration of the effect of temperature on the circulation in the hand, the glove must be surrounded by water, (2) in figure. Between the chamber *A* and the T-tube *D*, a 2-litre bottle *G*, with bottom tubulure, is introduced, *A* being connected with *G* by fairly large bore, *e.g.* 1 cm., rubber tubing about 1 metre in length.

The bottle is first filled with water, *A* is held mouth-downwards on the table; by alternately raising and lowering the bottle the air is displaced by water. To introduce the hand, *F* is closed, *E* is opened, and the bottle is lowered sufficiently below the level of *A* (which is now fixed horizontally on the table) to distend the glove, and the hand is introduced. The bottle is replaced on the table and the water level in it is adjusted through a clipped T-tube *H* on the connection with *A*, to the level of the highest point of the plethysmograph chamber. *F* is now opened and *E* is closed after blowing a little air into the bellows. The mean pressure on the hand will be about 5 mm. of mercury. With a bottle of ordinary proportions the maximum change in volume likely to be observed will only raise the level of water in the bottle about 2.5 mm. the pressure of which is negligible.

A can be cooled or warmed from the outside, a thermometer being cemented on to the inside in a visible position.

The plethysmograph chamber is fixed to the table with plasticine. The subject should sit in a comfortable position, the fore-arm and elbow being steadied by resting on a suitably adapted bag of sand or saw-dust.

The instrument has been used for measuring the volume of blood contained in the veins and the rate of venous engorgement as the result of (a) constriction of the arm by a sphygmomanometer band exerting various pressures, (b) sustained expiratory pressures.

A mercury manometer records the pressures in both cases.

The spread of the excitatory process in auricular muscle subjected to pressure¹. By A. N. DRURY.

It has already been shown by Lewis and Drury, *Proc. Physiol. Soc.* 1922, Feb. 18th(1), that if a region of pressure be interposed between the base and appendix of an auricle in the dog, and the auricle be driven rhythmically from the body, that the excitatory process may be delayed or prevented from reaching the tip of the appendix according to the pressure used.

In order to investigate the spread of the excitatory process in the compressed area, a special clamp was constructed composed of two blocks of ebonite. The top block 22 mm. in width was drilled with six small holes equidistant and in line, which were filled with kaolin and copper sulphate to form three pairs of non-polarisable electrodes. The galvanometric records thus obtained timed the arrival of the excitation process at three successive points under the clamp. The bottom block was grooved along the line of the non-polarisable electrodes, so that the muscle on each side was subjected to greater pressure, and the excitation wave thus forced along the line of the contacts. With the heart responding to rhythmic stimuli applied to the body of the auricle, it was found that the excitation wave penetrated the compressed muscle at each rhythmic stimulus, and that the rate of conduction became progressively slower as it passed further into the region of pressure. This was exemplified, with light grade of pressure, by the rate of conduction being quicker between the proximal and middle, than between the middle and distal contacts, with heavier pressure, by the appearance of a 2 : 1, 3 : 1 response or even complete block at the distal contact, a block of lesser degree at the middle contact and a response to each

¹ Observations undertaken on behalf of the Medical Research Council.

rhythmic stimulus at the proximal contact. The electrical records obtained showed definite and constant changes. As the rate of conduction became slower the amplitude of the record was much reduced and the speed at which the upstroke or downstroke of the intrinsic deflections were written much decreased. The records from the distal electrode were always more degraded than those from the middle, while those from the proximal retained in large measure their original form.

It would appear therefore that the lengthened transmission intervals reported in the journals quoted are produced by a progressive slowing of the excitatory process as it passes through the region of pressure. The 2 : 1 response of the tip is brought about by the failure of every alternate excitation wave to pass completely through this region; the complete block by the dying out of every wave in the compressed area.

Vagal stimulation, as has already been shown, relieves intra-auricular block produced by pressure. Using the clamp described, it can be shown that, during vagal stimulation, the excitatory process spreads more rapidly through, and penetrates further into the region of pressure, so that the lengthened transmission intervals are shortened and the higher grades of block relieved.

In compressed muscle, therefore, the excitation process is progressively slowed as it travels along and will finally die out if the degree of pressure be sufficiently heavy, or the width of the compressed area sufficiently great. If it is able to pass through the compressed region it enters normal muscle again, and the rate of conduction then approximates to its normal value.

Subjecting auricular muscle to pressure, therefore, calls forth phenomena similar to those observed in narcotised nerve, and there called "decrement."

(1) Fully reported in *Heart*, 10. p. 179. 1923.

Observations on the latent period of skeletal muscle.

By JOHN FARQUHAR FULTON.

The electrical and mechanical responses of the twitches of intact gastrocnemii (frogs) with active circulation have been recorded simultaneously on "fast" plates (50 cm. per sec.). Two myographs of the torsion-wire type have been employed, one having a natural frequency of 460 per sec., the other, 1600 per sec. Precautions have been taken to lighten the moving parts of the myograph, and the latency of the

recording system due to inertia is as low as 0.3σ and is probably much less. The muscle, stimulated through its nerve and attached to the myograph by a light steel hook, is placed before each response under 50 to 100 gms. initial stretch that time may not be lost in taking up "slack." Leads to the galvanometer are taken from the tendon, and from the belly of the muscle. By placing the proximal lead at varying distances (4 to 27 mm.) from the tendon lead, it has been shown that the most proximal portion of the muscle (at 20°) responds only 1σ before the most distal portion. One may infer from this that the response of the whole muscle taken with these precautions is an accurate (within $\pm 1\sigma$) expression of the responses of the individual fibres.



Fig. 1. Responses of the intact gastrocnemius of a decerebrate frog at 16.5° . Time indicated above, 0.02 sec. The horizontal shadows from above downwards are: the signal denoting the moment of the second stimulus (make); the myograph; the string of the galvanometer; signal for the first stimulus (break); line of zero tension, 29 mm. movement of the myograph vertically being equal to 500 gms. tension. String tension 5 mm. per m.v., the magnification being 285. Stimuli delivered to the cut nerve, the cathode being at a point 1.9 cm. from the entry of the nerve into the muscle; stimuli just-maximal induction shocks. Initial tension 90 gm. Frequency of the myograph 460 per sec. (The line of zero tension was cut off in reproduction; it may be taken as the lower margin of the plate.)

Gasser and Hill⁽¹⁾ have described, in the responses of the sartorius, a period of rigidity before shortening. To investigate this point further, it was arranged that a second stimulus should fall at the moment of "half-relaxation" from a first response. At an interval of 2.5 to 3.0σ after the electrical response of such a second stimulus the mechanical record shows a period of flatness which commences abruptly and lasts for 5σ at 20° . This has been interpreted as the period of rigidity of Gasser and Hill. Actual shortening begins at the end of the period of rigidity, and is of sufficient abruptness to form on the linear record a precise angle. The period of rigidity is greater for submaximal stimuli than for maximal. The period of true latency—from the electrical response to the beginning of rigidity—appears to be identical with Burdon Sanderson's⁽²⁾ latent period of thickening, and the results,

therefore lend no support to the view that the electrical and mechanical responses of skeletal muscle begin simultaneously.

In addition to the true latency there is also an end-plate delay, previously observed by Forbes, Ray and Griffith(3), which at 20° is approximately 3 σ . The latent period of shortening may therefore be divided into four phases: (1) transmission time of the nerve; (2) the delay at the motor end-plate; (3) the true latency; and (4) the period of rigidity. Each phase has a distinct temperature coefficient.

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- (1) Gasser and A. V. Hill. *Proc. Roy. Soc.* 96B. pp. 398-437. 1924.
- (2) Burdon Sanderson. *Journ. Physiol.* 18. pp. 117-159. 1895.
- (3) Forbes, Ray and Griffith. *Amer. Journ. Physiol.* 66. pp. 553-617. 1923.

PROCEEDINGS
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PHYSIOLOGICAL SOCIETY,
November 15, 1924.

A theory of sensory adaptation. By C S MYERS

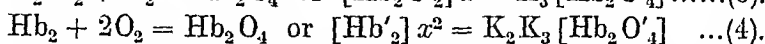
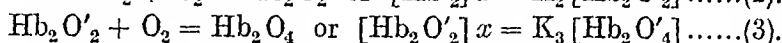
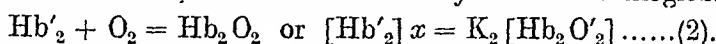
As an explanation of the phenomena of sensory adaptation and the like, no hypothesis has successfully supplanted that of Hering,—that certain pairs of opposite sensations, *eg* of warmth and coolness, or redness and greenness, arise from antagonistic reactions within a single sensory substance or mechanism. One of the difficulties in accepting this hypothesis has always consisted in the terms (of “dissimulation” and “assimilation”) in which such antagonism should be described. The only alternative hitherto suggested, so far as I am aware, to this improbable terminology employs the conception of reversible chemical action.

I wish to propose a somewhat different view, based on analogy with the reciprocal inhibition existing between extensor and flexor muscles during extension and flexion, and with the long continued, simultaneous extensor and flexor activity maintained during muscular tone and posture. Just as the act, say, of flexion involves simultaneous inhibition of antagonistic extension, so, I suggest, we may reasonably conceive that the development of the sensation say, of warmth, involves simultaneous inhibition of its antagonistic mechanism which would when excited develop the sensation of coolness. *Mutatis mutandis*, the same applies to the sensation of coolness, and to sensations of visual brightness and colour.

Further, just as a long continued muscular posture may be assumed in which both flexion and extension are simultaneously active, so, I suggest, a neutral position of sensory adaptation may be reached in which the mechanisms for warmth and coolness, redness and greenness, or the like, balance one another, whereupon no sensation of temperature or of colour arises.

Thus the mechanisms for warmth and coolness, redness and greenness, etc., may be in an active state of adaptation or “set”, or either member of the pair may be active while the other member is inhibited. According

the oxygen concentration, the following equations express the mass law relationships of the reduced, intermediate and oxy forms of hæmoglobin.



The hæmoglobin is represented as double molecules because as pointed out by Bohr the oxyhæmoglobin dissociation curve can be interpreted only when the square of the oxygen pressure is used (2).

The amount of oxygen (y) obtainable from a solution of hæmoglobin, on the assumption that the intermediate substance does not give off any of its oxygen is

$$y = [\text{Hb}_2\text{O}'_4] (1 + [\text{H}^+]/K_4) \text{ or } [\text{Hb}_2\text{O}'_4] = y/(1 + [\text{H}^+]/K_4) \dots\dots(5).$$

By substituting values from equations (2) to (5) in (1) we can deduce the following

$$y/100 = bx^2/(1 + ax + bx^2) \dots\dots\dots(6)$$

in which $a = (1 + [\text{H}^+]/K_5)/K_2 (1 + [\text{H}^+]/K_4)$

and $b = (1 + [\text{H}^+]/K_4)/K_2 K_3 (1 + [\text{H}^+]/K_1).$

We have applied equation (6) to a number of dissociation curves by the method of least squares and invariably the coefficient a is negative therefore the theoretical interpretation given above is inapplicable but the empirical equation fits a number of published curves.

Table showing application of $y/100 = bx^2/(1 + ax + bx^2)$ to the curves published by Bock, Field and Adair(1).

	x	= 10	20	30	40	50	60	70	mm. Hg.
y	Expt.	= 8	23	45	64	77	85	90	CO ₂ pressure, 80 mms.
	A. V. H.	= 6.6	26.9	49.5	66.1	76.8	83.7	89.1	
	R. & S.	= 6.8	24.7	45.6	63.1	75.9	84.0	90.5	
y	Expt.	= 15	40	60	76	85	91		CO ₂ pressure, 40 mms.
	A. V. H.	= 13.7	42.5	64.5	77.6	85.0	89.0		
	R. & S.	= 13.5	40.6	62.8	76.9	85.4	90.6		
y	Expt.	= 20	50	73	87	93	96		CO ₂ pressure, 20 mms.
	A. V. H.	= 17.2	54.6	77.0	87.3	93.9	95.1		
	R. & S.	= 18.5	51.4	73.9	86.2	92.7	96.4		
y	Expt.	= 40	80	93	97				CO ₂ pressure, 3 mms.
	A. V. H.	= 37.0	77.2	93.2	96.8				
	R. & S.	= 42.3	79.1	92.3	97.2				

Note:—A. V. H.=values calculated from Hill's equation (4) by method of least squares; R. & S.=our calculated values.

As pointed out by Bock, Field and Adair(1) the logarithmic form of Hill's equation namely

$$\log y/100 - y = n \log x + \log K$$

does not yield a straight line. The corresponding form of our equation

$$\log y/100 - y = \log x^2/(1 + ax) + \log b$$

when similarly plotted does yield a straight line, from which we see that

the amount of oxygen obtainable from oxyhæmoglobin increases more rapidly than it ought to do when calculated on a mass law basis. Henderson attributes this excess to the increased acidity of oxyhæmoglobin(3) contrasted with that of reduced hæmoglobin but we believe that the oxygen content as measured includes oxygen from some other source. This we are now investigating.

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- (1) Bock, Field and Adair *Journ Biol Chem* 59 p 353 1924
- (2) Bohr *Zentralblatt f Physiol* 17 p 682 1904
- (3) Henderson *Journ Biol Chem* 41 p 416 1920
- (4) Hill *Biochem Journ* 7 p 471 1913
- (5) Roaf and Smart *Ibid* 17 p 579 1923

"The Bohr effect" in the living animal. (*Preliminary Communication*) By J ARGYLL CAMPBELL

Bohr, Hasselbalch and Krogh(1) demonstrated that hæmoglobin gives off O_2 more readily in the presence of CO_2 , thus facilitating the liberation of O_2 in the tissues. Barcroft and Odell(2) showed that lactic acid has a similar effect.

I have found that moderately severe exercise in rabbits increases the O_2 -tension, as much as 10 mm Hg in an hour, in gas injected into the abdominal cavity and under the skin. This method of injection of air or N_2 to study changes in CO_2 and O_2 tensions in body cavities and tissue spaces has been described(3).

By simply grasping once every five seconds, one or other hind leg of a healthy rabbit, the animal is made to 'kick about' vigorously for four or five minutes, the exertion is of such a nature as to produce obvious hyperpnoea but not distress. The animal is then rested for about ten minutes, during which interval samples of gas are withdrawn for analysis from the abdominal cavity and from under the skin. The whole procedure is repeated three times, so that there are three short periods of moderately severe exercise each followed by a period of rest, the complete experiment lasting about an hour or so. This type of experiment was employed by Douglas and Haldane(4) to study the effects of severe muscular exercise upon the alveolar CO_2 tension in man. I find that the effects upon the CO_2 tension in gas in the abdominal cavity in rabbits are similar to those given by Douglas and Haldane for the alveolar CO_2 tension in man, that is to say, during each period of rest the CO_2 tension in gas in the abdominal cavity at first increases and then markedly decreases but in each period the tensions are lower than in the

preceding period, because as the number of periods of exercise increases the amount of lactic acid in the blood and tissues also increases. The effects on the CO_2 -tension in gas under the skin are similar to the above, but the CO_2 -tension under the skin remains high for a longer period. In any case both in the abdominal cavity and under the skin during the third period of rest the CO_2 -tension is, owing to the accumulation of lactic acid, reduced below normal by about 15 mm. Hg, *e.g.* from 50 to 35. At the same time the O_2 -tension is increased by as much as 10 mm. Hg, *e.g.* from 22 to 32 under the skin and from 38 to 48 in the abdominal cavity; this indicates a rise of O_2 -tension throughout the tissue spaces of from 25 to 50 p.c. This is, of course, far too great a rise to be due to the small fall in total volume of injected gases by disappearance of about 2 p.c. CO_2 . It is not due to dilation of blood vessels since a *general* dilation causes from the start a fall of O_2 -tension, *e.g.* after histamine or during artificial respiration(3). The rise of O_2 -tension appears to be "the Bohr effect"; the accumulation of acid products in the tissues and blood increases the liberation of O_2 - from hæmoglobin so that the O_2 -tension rises in the gas in the abdominal cavity and under the skin. It may remain above normal for many hours, *e.g.* 24. During the earlier part of the experiment the O_2 -tension may fall 1 or 2 mm. Hg, or remain unaltered.

The results are independent of the volume of gas present within the wide limits tested, that is, 50 to 500 c.c. under the skin and 50 to 250 c.c. in the abdominal cavity.

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- (1) Bohr, Hasselbalch and Krogh. *Skand. Arch. f. Physiol.* 16. p. 402. 1904.
- (2) Barcroft and Orbeli. *Journ. Physiol.* 41. p. 353. 1910.
- (3) Campbell. *Ibid.* 59. p. 1. 1924.
- (4) Douglas and Haldane. *Ibid.* 38. p. 430. 1909.

A note on the foetal and maternal blood-sugar at birth.

By NORMAN BURGESS.

This work was undertaken with a view to comparing the foetal blood-sugar with that of the mother at the time of birth, and to finding some indication of the relations between them.

The blood-sugar was estimated by the method of Folin and Wu. Six cases of normal labour were investigated, the blood being taken from the finger of the mother and from the placental end of the divided umbilical cord, immediately after the delivery of the child. It seems

unlikely that the delay in the foetal circulation caused by this method would have operated for a sufficient length of time to have any great effect on the blood.

The results obtained, together with some clinical details of each case, are shown in the following table:

Case	Maternal blood-sugar p.c.	Foetal blood-sugar p.c.	Duration of labour	Position of child	Condition of mother, etc.
I	0.154	0.135	3 days	Left occipito-anterior	Primipara. Aet. 29 yrs. Pulse 80. Temp. 99.6°
II	0.125	0.095	6½ hrs.	Left occipito-anterior	Multipara. 2nd pregnancy. Aet. 24 yrs. Pulse 80. Temp. 97.4°
III	0.117	0.118	7 ..	Right occipito-posterior	Multipara. 2nd pregnancy. Aet. 28 yrs. Pulse 62. Temp. 98°
IV	0.13	0.11	7 ..	Left occipito-anterior	Multipara. 2nd pregnancy. Aet. 27 yrs. Pulse 82. Temp. 97.6°
V	0.13	0.101	6 ..	Right occipito-anterior	Multipara. 4th pregnancy. Aet. 26 yrs. Pulse 68. Temp. 98.4°
VI	0.07	0.11	4½ ..	Right occipito-anterior	Multipara. 7th pregnancy. Aet. 38 yrs. Pulse 108. Temp. 98°

From the figures obtained it would appear that severe and prolonged labour causes an increase in the maternal blood-sugar, since in Case I where the labour lasted three days, the maternal blood-sugar reached the highest level; also in four out of the six cases investigated, the maternal blood-sugar is higher than the normal resting figure (0.1 p.c.). It seems probable that muscular activity and increased suprarenal secretion would account for this. With regard to the foetal blood-sugar¹ this appears more stable. In four out of the six cases it is lower than the maternal. Case I shows that when the maternal blood-sugar is much raised, the foetal blood-sugar is also raised. In Case VI an abnormally low maternal blood-sugar was found. The cause of this is not clear but many cases have been described where the blood-sugar has normally been at a low level.

In this case the foetal blood-sugar preserved a normal level.

Work is now being carried out in order to discover the effect of exercise on the blood-sugar level and on sugar tolerance, and this should help us to a clearer understanding of the observations recorded here.

¹ I.e. Reducing sugar, since it is known that laevulose is present in the blood of the foetus.



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A simple method of recording the respiratory movements.
By THOMAS LUMSDEN.

Respiratory tracings as commonly taken by means of a Y-shaped tracheal cannula are unsatisfactory. Such tracings do not give accurate information as to the extent of the respiratory movements, and when the chest is quiescent they do not indicate whether the pause is in the inspiratory or the expiratory position. The best and simplest method for general use is to strap one glycerine tambour (of about two inches diameter and half-an-inch deep) on the chest and another on the abdomen. Both are necessary, since the former does not record expiratory spasms if of moderate intensity, and the latter does not always show gasps. Each tambour has two rubber membranes with glycerine between them. To prepare such a tambour fix a pressure tube on its nozzle, tie on the inner diaphragm, then suck out the air and clamp the tube, the rubber membrane is now concave; fill the concavity with glycerine and tie on the outer membrane firmly. When the tube is released the air re-enters, and the outer rubber membrane becomes markedly convex. Each tambour communicates by light pressure tubing, and forms an air-tight system, with a smaller ordinary tambour of appropriate capacity bearing a writing lever. Thus during inspiration as the chest expands, it presses on the thoracic tambour driving air from it into the corresponding recording tambour, proportionately raising the writing point and keeping it raised as long as inspiration lasts. The abdominal tracing requires careful interpretation as previously explained (1).

The tambours can be got from Hawksley of 83 Wigmore St., W. 1, or be made in any laboratory.

The only technique I know of, which can compare in accuracy with the simple method described above, is to take tracings direct from both inspiratory and expiratory muscles. The latter is always a very troublesome procedure and the former is easy only in rabbits and hares (Head's

method), animals which are in other ways much inferior to cats in respiratory experiments. The glycerine tambour method gives tracings which are proportionate in time and in degree to the respiratory movements both inspiratory and expiratory. It does not require tracheotomy or other operative interference, and is therefore applicable to the human subject. It is the simplest way of recording apneuses and expiratory tetanic movements.

(1) Lumsden. Journ. Physiol. 57. p. 354. 1923.

Effects of bulbar anæmia on respiratory movements.

By THOMAS LUMSDEN.

In previous papers(1) I gave evidence that the central mechanism of respiration consists of four separate but coordinated centres. One part of that evidence given in my second paper, p. 359, was that in progressive anæmia of the brain stem these centres went out of action separately and in order from above down, viz. (1) Pneumotaxic, (2) Apneustic,

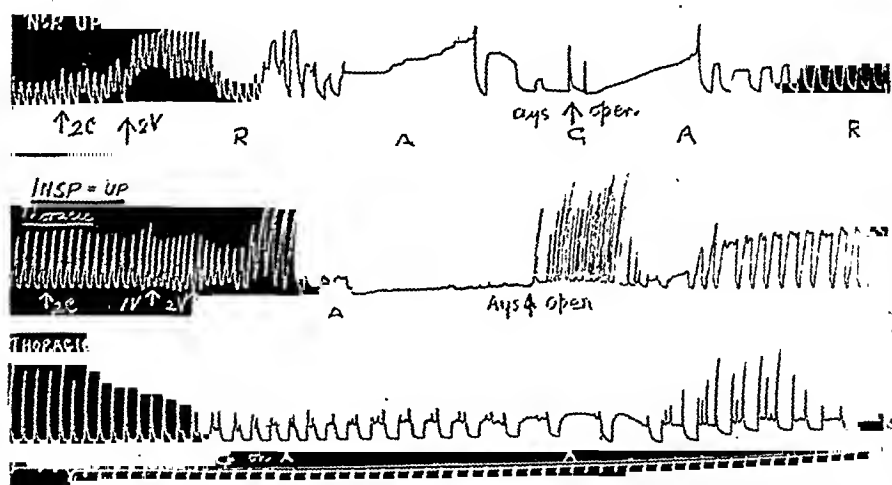


Fig. 1. Thoracic tracings (cats). Insp. up. Top line=*a*, middle=*b*, see text; lowest=*c*, gradual revival of apneusis inhibiting gasps, later failure of apneusis releasing gasps. Pneumotaxic centre dead. Time, 5 secs. 2C=carotids closed, 2V=vertebrals.

(3) Expiratory, and lastly (4) Gasping centre. In a recent paper(2), Roberts failed to confirm this and stated that he never observed apneusis either during production of cerebral anæmia, or recovery from it. That he should fail to do so was almost inevitable since he omitted the following necessary precautions: 1. The use of adequate apparatus,

capable of recording inspiratory touns (apneusis) or expiratory spasms. It is clearly impossible to record these by means of a Y-shaped tracheal cannula such as Roberts generally used. 2 Cutting the vagi if dogs or rabbits are used. 3 Maintaining the anaemia till gasping appears or till the B.P. falls to 20-30 mm. 4 The cerebral blood-pressure should not be taken when studying respiratory changes.

An effective method of recording respiration is described elsewhere (3).

The blood may be shut off from the brain stem by the method Roberts used or in a young cat (under six months), the carotids may be clamped and the vertebrals digitally compressed at the level of the atlas.

I have repeated my experiments by each of these methods, the results (Fig. 1) which invariably confirmed my previous observations may be summarised and accounted for as follows:

1. A brief period (20-30 secs) of excitation occurs, with increased

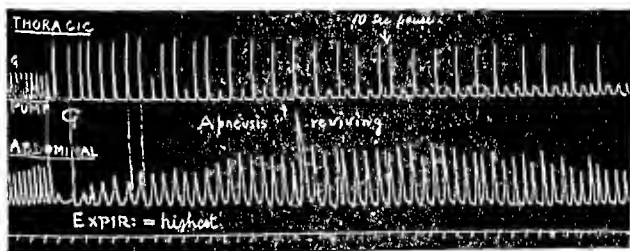


Fig. 2 Cat. Top=thoracic, lower=abdominal. Recovery after artif. resp. First gasping next expiratory spasms (abdom. tracing), then inhibition of gasp and apneustic revival (top). Time, 5 secs.

pulmonary ventilation. 2 From lack of O_2 the pontine (pneumotaxic) centre first fails, inspiratory touns are no longer inhibited and apneusis appears. 3 The apneustic centre next fails, a few gasps occur, then breathing ceases. If the arteries are now freed, respiration is restored (after artificial respiration if necessary) in the reverse order. In a rabbit or dog with intact vagi or in a cat after too brief anaemia, apneusis may not be observed, since it is at once periodically inhibited. The increasing respiration so produced gradually replaces gasping. During very slow recovery the revival of the separate centres in order from below upwards is well seen, Fig. 1 (c) and Fig. 2. In some cases the pneumotaxic centre does not revive, apneustic respiration then goes on for an hour or more,

Fig. 1 (c). It is difficult, unless the chest is opened (see second paper, p. 356) to observe apneusis visually, an adequate tracing is essential. When gasping and respiration co-exist their rhythms roughly harmonise. This is chiefly because the impulses from the apneustic and gasping centres pass by a common path to the same muscles.

The facts then demonstrate the existence of the four separate respiratory centres previously located on the invariably consistent results of over 500 sections of the brain stem.

(1) Lumsden. *Journ. Physiol.* 57. pp. 153, 354. 1923; 58, pp. 81, 111. 1924.

(2) Roberts. *Ibid.* 59. p. 99. 1924.

(3) Lumsden. *Proc. Phys. Soc.* 59. p. lvii. 1925.

The action of sparteine sulphate on experimental fibrillation of the auricles. By J. HAMILTON CRAWFORD.

Considerable interest has been shown in the treatment of auricular fibrillation since it was shown that quinidine sulphate could cause a reversion to normal rhythm. It seemed likely that this property was not peculiar to cinchona alkaloids but was common to all substances which had a depressor action on cardiac muscle. Sparteine sulphate had been stated by previous observers to depress the muscle of the heart, and we have recently confirmed their findings. Consequently it seemed a suitable drug to use in order to test the above hypothesis. At the time these experiments were in progress studies on the pharmacology of sparteine were also being made by Bohnenkamp and Hildebrant⁽¹⁾ who have recently published their results. In one study they produced irregularity of the heart by means of strophanthin. Afterwards they gave sparteine sulphate and state that the animal visibly improved and remained alive. They suggest that sparteine may have an action similar to quinidine. They do not appear to have taken graphic records of this part of their work.

In my experiments dogs anaesthetized with morphine and intramuscular chloretone were used. The movements of the auricles and ventricles were recorded by means of Cushny's myocardiograph, and auricular fibrillation was produced by faradic stimulation of the auricle. The stimulus was found which was necessary to produce auricular fibrillation and this was continued during the period the drug was in action; on some occasions a stronger stimulus was used than the minimum necessary to produce fibrillation. Sparteine sulphate was injected into the jugular

vein as a 1 p.c. solution in doses varying from 5 to 50 mg. per kilo. In every case an effect was produced; with small doses definite auricular beats appeared although there was still marked irregularity of both the auricle and the ventricle. Larger doses caused more numerous auricular beats to appear until a stage was reached when the auricle beat rapidly but quite regularly. The ventricle at this stage was still irregular, but with increasing dosage the auricles and ventricles both became regular although still more rapid than normal; the rhythm then was either 1-1 or 2-1. Finally, still larger doses caused complete return to normal rhythm with both the auricle and ventricle beating at a slower rate than before stimulation was applied. When this change took place, the heart responded to the sino-auricular node and not to the faradic stimulation. Strengthening the stimulus increased the rate of both the auricles and ventricles but produced no reversion to the fibrillating state. A definite dose produced a more marked effect when given in one injection than when the same quantity was given in divided doses.

(1) Bohnenkamp and Hildebrandt. *Arch. f. exper. Path. u. Pharm.* 102. p. 244. 1924.

Recovery from a period of malnutrition in the growing animal.

By V. H. MOTTRAM and G. HARTWELL.

Recovery from a period of malnutrition is fairly rapid in the infant. Thus a child who has ceased to grow for 10 days owing to under-nutrition will make good both the loss and the otherwise probable gain in 16 days and the result of a period of malnutrition lasting 5 weeks may be obliterated in as little as 6 weeks.

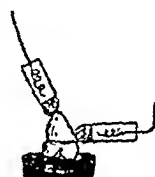
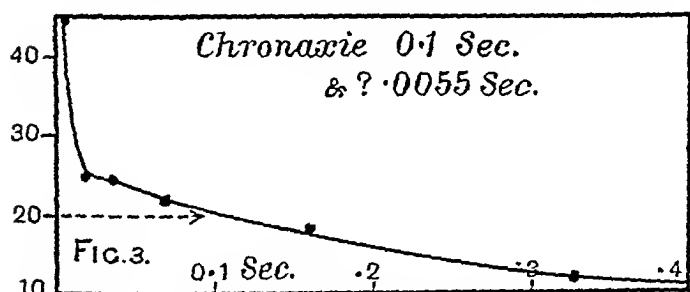
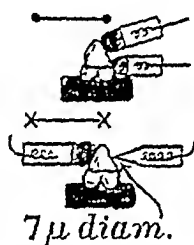
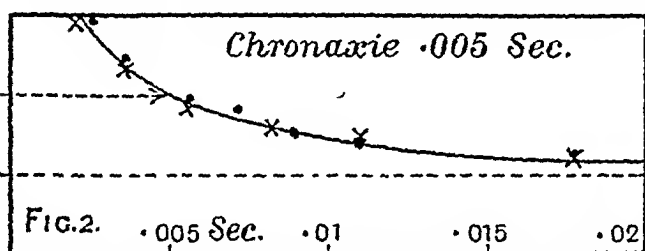
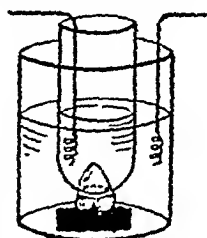
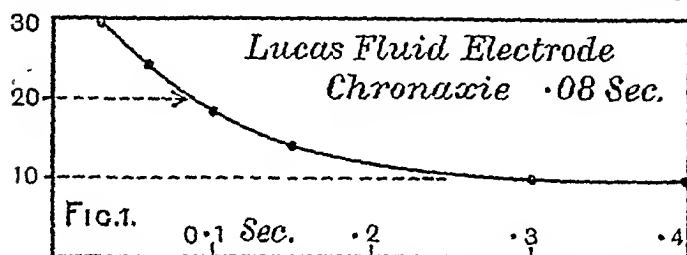
With growing rats it is otherwise. A period of a few days' malnutrition during the nursing period, with the mother on a poor diet, has such an effect that the acceleration of growth when she is on a good diet does not enable the young to make up the leeway. Similarly with rats after weaning. 13 days malnutrition, due to a deficit of quantity and quality of protein, but of nothing else, produces such a retardation of growth that it is 7 weeks before the animals approximate to the controls in weight, though on a diet which produces optimal rate of growth. Restitution of proteins to a diet has a different effect from restitution of vitamin.

The probable explanation of the difference is that a day is so much greater a percentage of a rat's life than of a human being's. 13 days is 1.2 per cent. of a rat's life; 5 weeks but 0.14 per cent. of a child's. 13 days is about one-tenth of the rat's growing period, 5 weeks one two-hundredth of a child's.

The chronaxie of frog's ventricular muscle.

By E. D. ADRIAN.

The time factor for the excitation of cardiac muscle has been recorded several times, but, as with skeletal muscle, there is a serious disagreement between the strength-duration curve given by Keith Lucas and the measurements of the chronaxie by the method of Lapicque. Lucas(1) gives two curves, typical of five more, for the frog's ventricular muscle in which the minimal current strength must be doubled when the duration is reduced to 0.2—1.0 sec. M. Lapicque and Veil(2) give .003—.0035 sec.



as the chronaxie for mammalian ventricular muscle, M. Lapicque(3) gives 0.7 mf. (about .005 sec.) for the frog and Fredericq(4) .006—.014 sec. for the tortoise. Recent work(5) has shown that in skeletal muscle the type of electrode has a great influence on the chronaxie, shorter values being found when the area of the cathode is small. The strength-duration curve has therefore been determined for the frog's ventricle with various electrodes, the current being delivered by a mechanical contact breaker. With the Lucas electrode (Fig. 1) the chronaxie is invariably long,

between .08 and .5 sec. With other electrodes, however, the curve nearly always gives the short value (.005 sec.) and this holds good for a Pratt electrode with a pore of 5μ or 70μ , for electrodes ending in threads, or in large cotton wool pads (Fig. 2). Thus the factor of electrode area alone does not affect the curve. The direction of current flow, from base to apex or at right angles, is also without effect. There remain two peculiarities of the Lucas electrode which might account for the long value. One is that the current density is more or less uniform over the entire aperture so that the stimulus might take effect on a structure of long chronaxie inside the ventricle. A long value, or a complex curve rising at long durations is occasionally found with large pad electrodes on either side of the ventricle (Fig. 3). The structure can scarcely be the auriculo-ventricular junction. Lapicque finds that this has a chronaxie 2 or 3 times as long as that of ventricular muscle, but the chronaxie given by the Lucas electrode in Fig. 1 is 16 times as long as that in Fig. 2. Another possibility is that the long value depends on the peculiar distribution of current flow in the Lucas electrode and in particular on the close approximation of anode and cathode. Pointed electrodes 1 mm apart do not give a long value, but this was sometimes found when the electrodes formed parallel strips 1 mm apart. Whatever the explanation, it seems that the short chronaxie (.005 sec.) is more truly representative of ventricular muscle, and that in this tissue the factor of electrode area by itself is not important in fixing the time constants of the curve.

(1) Keith Lucas Journ. of Physiol. 39 p. 471 1910

(2) Lapicque and Veil Compt. rend. Soc. Biol. 91 p. 368 1924

(3) Lapicque Compt. rend. Acad. Sci. 161 p. 103 1916

(4) Fredericq Bull. Acad. roy. med. Belg. 4 p. 481 1924

(5) Cf. Watts Journ. of Physiol. 59 p. 143 1924

On the effects produced by the assimilation of large amounts of glucose. (*Preliminary Communication*) By T. IZOD BENNETT and E. C. DODDS

We have administered glucose in solution in single doses of half a pound or more to a large number of healthy individuals, laboratory workers and medical students, glycosuria of a trivial nature occurred in about 30 per cent of all subjects, the total sugar lost in all cases aggregating to less than 1 gm. In three subjects an amount equal to 500 gm. was administered in one dose, in one subject only did glycosuria follow, this being in so small amount that total sugar lost in urine could not be

calculated. Observations on urine, expired air, blood and stomach contents were made on these three subjects before and at intervals after the ingestion of glucose, with the following results:

1. *Urine.* In two of the three subjects, there was marked diuresis for the first hour and a half, the urine being free of sugar and of low specific gravity; the third subject showed no diuresis but passed traces of sugar half an hour and one hour after taking the glucose.

2. *Expired air.* The expired air collected before and $1\frac{1}{2}$ hours after glucose ingestion showed no appreciable change in the respiratory quotient.

3. *Blood.* Blood-sugar estimations were made on all three subjects at half-hourly intervals; these showed a rise in blood-sugar from a fasting level of 83-90 mg. p.c., to 114-125 mg. half an hour after glucose ingestion, with a steady return towards original level in subsequent specimens. The rise of blood-sugar amounted to 40 mg. in one subject only, this was the subject who exhibited a trace of sugar in the urine.

Estimations of blood-urea, non-protein nitrogen, uric acid and creatinin in the same specimens showed a diminution in the concentration of these substances in the blood after glucose ingestion.

4. *Stomach-contents.* Specimens of gastric contents withdrawn at half-hourly intervals showed a progressive diminution in the concentration of glucose solution in the stomach; some glucose remained in stomach $2\frac{1}{2}$ hours after administration.

General effect. In two of the three subjects an unexpected effect was observed. This was a condition of deep somnolence which occurred 3-5 hours after taking the glucose. It was observed independently by the two subjects and cannot have been due to suggestion. Both subjects exhibited absence of appetite, but no distaste for food at mid-day, and immediately after were overcome by sleep which lasted for some three hours.

Our results confirm those of Taylor and Hulton(1) showing that the ingestion of 500 gm. of glucose does not lead to any appreciable loss of sugar in the urine of healthy subjects.

Our observations on stomach-contents, urine and blood suggest:

(a) That strong glucose solutions are retained and diluted in the stomach for some time before complete absorption.

(b) That there is a general dilution of the blood by tissue fluids when such solutions are taken.

(c) The relative splanchnic hyperæmia may account for the hypnotic effect.

No explanation of the initial diuresis, which in one subject amounted to over one litre, appears satisfactory. These observations throw doubt on the current belief that an "increased tolerance," such as is supposed to occur in conditions of pituitary insufficiency, can occur. The normal tolerance for glucose appears to be limited by the appetite alone.

(1) Taylor and Hulton. *Journ. Biol. Chem.* 25. p. 173. 1916.

The coagulability of hæmophilic blood. By J. W. PICKERING
and R. J. GLADSTONE.

Opinions differ widely respecting the delay of blood clotting in hæmophilia. It was attributed by Sahli⁽¹⁾ to a deficiency of thrombo-kinase, by Howell⁽²⁾ to a shortage of pro-thrombin, by Addis⁽³⁾ to an altered state of pro-thrombin and by Feissly⁽⁴⁾ to an inhibitory substance in the plasma and serum. Most observers are, however, agreed that no excess of anti-thrombin is obtainable from hæmophilic blood and that no abnormalities are apparent in the platelets, thrombin and fibrinogen. We have investigated two hæmophilic bloods: (1) from a severe case in which clotting, on glass at 16°, was delayed an hour, and (2) from a milder case in which periodic epistaxis occurred and the clotting times, at room temperatures, varied from 16 to 20 minutes. In both cases the shed blood, if kept at 40° on clean glass surfaces, clotted firmly in from 6 to 8 minutes. At 16° the addition of either a hæmophilic clot or serum induced clotting in 5 minutes 30 seconds and 3 minutes 10 seconds respectively. When blood from the severe case was mixed immediately, at 16°, with an equal volume of normal human blood clotting was 7 minutes slower than that of normal blood. The blood of the milder case similarly inhibited the clotting of normal blood, but more was required than when blood from the severe case was used. When the mixtures were made 4 minutes after the normal blood had been shed, no inhibition of clotting occurred. Hæmophilic blood thus restrains the inception of blood clotting, but does not delay the later stages of coagulation.

We also found that the blood of eleventh-day chick embryos, when added immediately to an equal volume of normal human blood, delays its clotting from 40 to 60 minutes. Like hæmophilic blood, it does not inhibit the later stages of coagulation. The protective material appears to be a globulin, which coagulates at 74°-75°. It is noteworthy that Hurwitz and Lucas⁽⁵⁾ found a relative excess of globulin in hæmophilic serum. The protection of embryonic blood against the inception of

clotting decreases with the ageing of the embryo and is absent in adult fowls' blood, which clots at 40°.

It appears probable that the delay in clotting in the hæmophilic bloods examined is due to a persistence in adult life of an embryonic condition of the plasma and that in both hæmophilic and embryonic blood there is present an excess of a stable protective colloid. This conclusion is concordant with the theory of the fluidity and clotting of blood enunciated by one of us (J. W. P.).

- (1) Sahli. *Zeit. klin. Med.* 56. p. 264. 1905.
- (2) Howell. *Arch. Intl. Med.* 13. p. 76. 1914.
- (3) Addis. *J. Path. Bact.* 15. p. 427. 1911.
- (4) Feissly. *Schweizer. med. Woch.* 54. p. 81. 1924.
- (5) Hurwitz and Lucas. *Arch. Intl. Med.* 17. p. 543. 1916.

Reflex hyperglycæmia. By B. P. BABKIN.

In cats and dogs each operative interference highly increases the hyperglycæmia provoked by anæsthetics. The degree of hyperglycæmia corresponds to the gravity of the operation performed. The previous diet influences the blood-sugar content of the operated animal. By using special diet (milk and sugar) one can have an animal in an "acute" experiment with practically constant blood-sugar. Reflex hyperglycæmia may be produced in a decerebrated animal by stimulating the central end of the sciatic nerve. The analysis of this phenomena gave the following results: Removal of the adrenals and section of the splanchnics diminish the reflex hyperglycæmia but do not abolish it. Stimulation of the peripheral end of a motor nerve does not increase the blood-sugar content as also the stimulation of a sensory nerve in an eviscerated animal. Therefore the reflex hyperglycæmia which occurs as a result of stimulation of the central end of a sensitive nerve, both splanchnics and vagi being cut and both adrenals being removed, can hardly be ascribed to the conversion of muscle glycogen into the sugar. On the other hand, stimulation of vagi in the neck, the nerves being cut in the chest under the heart, or compression of inferior vena cava above the diaphragm, increases the blood-sugar content. Thus the changes of blood-pressure in the abdominal viscera—positive (stimulation of sciatic) and negative (stimulation of vagus)—seem to play a certain part in the mechanism of the reflex hyperglycæmia.

PROCEEDINGS

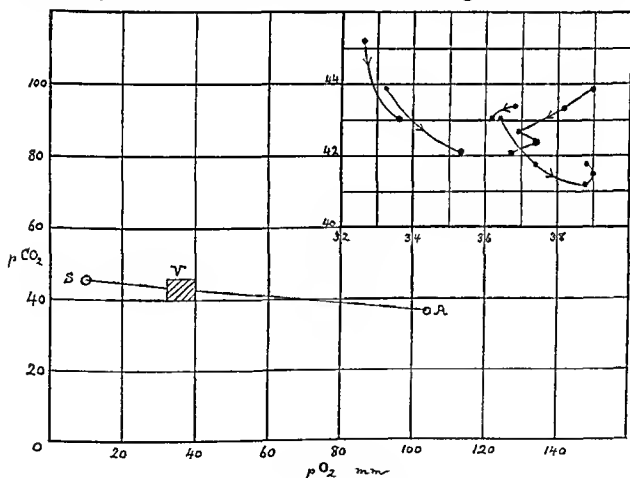
OF THE

PHYSIOLOGICAL SOCIETY,

January 17, 1925.

A method for the determination of the oxygen and carbon dioxide tensions in mixed venous blood. By CECIL D. MURRAY and HAROLD TAYLOR.

The pressures of oxygen and carbon dioxide in the mixed venous blood can be determined directly by gas analysis and, we believe, with accuracy by the method to be described. The figure illustrates the



principle of the method, and the inset gives results obtained on one individual as an example.

Equipment. A rubber bladder (cap. about 2.5 l.) is attached to the far end of a large-bore rubber tube (2.5 cm. dia., 1 m. long), and to the other end is attached a mouth-piece, near which the tube is pierced

to receive not more than five gas-sampling tubes. Just prior to use the bladder is filled from a stock gas mixture composed of approximately 7 per cent. CO_2 , 1.5 per cent. O_2 , 91.5 per cent. N_2 , and the tube then clamped.

Procedure. The subject, with nostrils clipped, expires completely. (The point *A* on the chart represents the composition of alveolar air—the residual air is perhaps slightly more “venous.”) Taking the mouth-piece, the subject inspires the gas mixture to the normal chest position, thereby collapsing the bag. (The stock mixture, point *S*, and the residual air on mixing in the lung yield a not necessarily uniform mixture with a composition falling within the venous area, the rectangle marked *V*.) The subject then expires completely—back into the bag—and the first sample is taken just as in sampling for alveolar air. The air is re-breathed, and at the end of each expiration a sample is collected until five in all have been taken. The experiment should be completed within 12 or 15 seconds. The samples are then analysed for O_2 and CO_2 .

The inset is a ten-fold enlargement of the venous area of one of us, and shows two five-point curves taken on two different days, and three two-point determinations taken within twenty minutes of each other on a third day. The two-point determinations are based on analysis of expirations 1 and 4, the other three expirations not having been sampled.

For purposes of calculation or definition we recommend using the results of sample 3 or of sample 4 or their mean. The evidence that we have here a measure of the true venous point rests upon the fact that out of twenty five-point curves on two of us, points 3 and 4 have always been identical within a limit of 1 mm. of both CO_2 and O_2 , and in the majority of cases point 5 has been included in the same mm. square with 3 and 4. (Point 5 usually indicates a trace of re-circulating blood). Moreover, the experiments which are given in the inset include all those that have been performed on one of us under presumably constant conditions, namely sitting in a low chair for ten minutes after a considerable period of ordinary laboratory activity; and these do not exhibit any greater variability than might reasonably be expected. Precautions such as would be taken for determinations of alveolar air or of basal metabolism should be observed by the subject prior to the experiment. Incidentally we find that the sitting posture is a more satisfactory standard than the reclining position, perhaps because of greater ease in breathing.

The difficulty which previous methods of determining the venous

point have not overcome is the high percentage of O_2 in residual air. Hence re-breathing gas mixtures previously made up to approximate the venous point involves starting toward equilibrium from a mixture which is really about half-way between alveolar air and "venous air." Such a method was tried by Douglas and Haldane(1), but was abandoned in favour of another method which necessitated elaborate knowledge of the subject's blood. Other methods have required similar knowledge of the blood, or they have relied upon extrapolations which are known to be only rough approximations.

NOTE: Since delivering this paper we have seen the article by C. S. Burwell and G. C. Robinson in the new *Journal of Clinical Investigation*, 1 p. 47, 1924. They describe a method which is similar to ours in principle, but appears unnecessarily elaborate. Their analyses are based on the gas in the bag instead of alveolar samples, hence several re-breathing periods are required, on the other hand they collect thus a quantity of gas to equilibrate with blood.

(1) Douglas, C. G. and Haldane, J. S. *Journ of Physiology*, 56 p. 60, 1922.

The maximum of human power, and the fuel of muscular work.

(From Observations on the Olympic Championship Crew of 1924.)

By YANDELL HENDERSON and HOWARD W. HAGGARD

Rowing in a racing shell with sliding seats probably allows a nearer approach to maximal work, by more nearly all the muscles of the body, than any other form of athletics. The eight men who rowed in the Yale University boat in 1924 demonstrated in a series of races, ranging from a mile and a quarter up to four miles, that for all these distances they were able to lead any other crew in their own country. After winning the right to represent America in the Olympic games at Paris, they won in the trial heats and led by several lengths in the final race on the Seine, winning from crews from all parts of the world and establishing a world's record for the 2000 meter course.

During the season of training from January to June 1924, we were fortunately able to make from time to time on five of these men determinations of the respiratory exchange and quotient, the oxygen consumption and deficit, the CO_2 output, etc. The external work was also determined by means of a rowing machine set up in the laboratory and arranged as an ergometer. Later a determination of the power necessary to drive a racing boat at various speeds was obtained by towing it by means of a power boat with the tow-line fastened to a spring balance. The figures from the draw bar pull multiplied by the speed in feet per minute give the absolute net work which the crew had to do. An additional 25 per cent of external work was assumed, in order to cover the energy expended in moving the slide and oar in returning to the

stroke position, and was added to the results from the rowing machine and draw-bar pull methods.

The data from these three methods were in general in fair agreement. They indicate that the maximal power exerted is from 0.45 to 0.55 horse power per man, or expressed in the heat equivalents, 4.8 to 5.9 calories per minute, with a total energy expenditure of 19 to 29 calories per minute, or 13 to 20 times the basal rate. The power expressed by the smaller of each of these pairs of figures is that maintained, and is therefore approximately the maximum that a man can maintain for 22 minutes during a four mile race; while the higher figures are applicable to the more intense exertion and greater speed, which are also maximal for about six minutes in races of about one and one-third miles or 2000 meters. The corresponding figures for the volume of oxygen consumed per minute are 3.5 and 4 litres; the latter figure is about the limit of the transporting capacity of the lungs, blood, and heart. An oarsman exerts a power which exceeds by 30 to 60 per cent. that afforded by the oxygen simultaneously absorbed; he thus draws heavily on his credit, and incurs oxygen deficits of 4 to 8 litres or more, and these deficits are repaid by the high rate of oxygen absorption for a time after the work is ended. This is in accord with A. V. Hill's conception.

The most significant result of these observations is the evidence, which they afford, in general agreement with Krogh and Lindhard, but in disaccord with the Hill-Meyerhof conception in its original form, that in whatever proportion fat and sugar are being burned during rest just before the exercise, they are burned in nearly the same proportion to produce the energy for doing work and for recovery. Thus it is found that in these oarsmen the respiratory quotients for the work and recovery periods combined were approximately the same as those during rest before the work. In one experiment the man had missed his breakfast and eaten nothing for eighteen hours; yet he made an intense exertion, although rather disadvantageously, on a combustion almost entirely of fat from his own body. His respiratory quotient during rest was .75, during three minutes of rowing on the machine it was .72, and during ten minutes rest afterward .73. The work done was equivalent to 6 calories per minute, the total energy expenditure 29 calories per minute; this man's weight was 180 pounds (82 kilos) and his height 6 feet 4 inches (193 cms.). In general the observations show that much more than half the energy expended by these athletes in muscular work is drawn from fat, and much less than half from sugar. A much larger proportion of carbohydrates would probably be advantageous.

In contrast to the effects of great exertion on untrained men, there was in the members of this crew only a slight overbreathing, or sometimes practically none at all, with a correspondingly slight blowing off of CO_2 during work or afterward. Apparently some of the phenomena, especially the blowing off of CO_2 and the high respiratory quotient during and immediately after intense exertion, which are commonly explained as due to the development of an "acidosis," and which Hill has instanced as in accord with the Hill-Meyerhof conception of muscular contraction, are due to the stimulation of the respiratory nervous regulation by oxygen deficiency in the arterial blood rather than to displacement of carbonic acid from the blood carbonates of lactic acid. (The full data will be published in the *American Journal of Physiology*.)

Determination of the circulation rate in man by inhalation of ethyl iodide. By YANDELL HENDERSON and HOWARD W. HAGGARD.

Ever since Harvey showed that the blood circulates, the determination of the volume of flow per minute has remained the outstanding unsolved problem of the circulation. The unsatisfactory condition of knowledge on this matter, and the importance of a simple, and fairly accurate method for measuring the circulation in man, has been pointed out by Y. Henderson in *Physiological Reviews* (vol. 3, p. 165. 1923). The method based on absorption of nitrous oxide from the lungs (Krogh and Lindhard) is extremely inaccurate and unreliable. The application of the Fick principle (the volume of oxygen absorbed from the lungs divided by the difference in the oxygen content of the arterial and venous blood) is too elaborate for use except in research. A simpler method has been sought for twenty years past in this laboratory along a wide variety of lines, and always in the end unsuccessfully.

Investigations in this laboratory during recent years have led to the formulation of the principles controlling the absorption of any gas whatever by mere solution from the lung air into the blood. (Haggard, *J. Bio. Chem.*, vol. 59, p. 753. 1924.) These principles show that the rate of absorption of a very soluble gas (e.g. ether or alcohol vapour) is dependent mainly on the minute volume of breathing, while that of a relatively slightly soluble gas is more nearly proportional to the blood stream. Gases of about the proper solubility were therefore tried, one after another, until the vapour of ethyl iodide was tested. With this substance an effective solution of the problem seems to be possible and the develop-

ment of a simple and fairly accurate method is probably only a matter of details of procedure.

The advantages of ethyl-iodide vapour are as follows: (1) Minute amounts of this substance, when vaporized in air, are analyzable very accurately by the iodine pentoxide method; and very low concentrations of the vapour may be used. (2) The distribution coefficient of the vapour between air and blood at body temperature is a conveniently low value, approximately 2.1. (3) The substance is not decomposed in the blood, but is decomposed so nearly completely in the tissues that the content of the venous blood returning to the lungs may be taken constantly as approximately zero, or at most as only about 5 per cent. of that in the arterial blood. Thus when air to which the attenuated vapour of ethyl iodide has been added is inhaled for a considerable period, 20 minutes, or more, and the expired air is sampled from time to time during this period, these samples are found to have a uniform concentration; showing that no accumulation in the body occurs. During rest the expired concentration is about half the inspired. (4) No co-operation on the part of the subject is necessary, other than to breathe as naturally as possible through a mouthpiece and valves. A preliminary period of two minutes is allowed for the concentration in the lungs to become uniform, and then the expired air is collected for three minutes.

The factors determined are: (1) the volume of air breathed per minute, (2) the content of ethyl iodide in the inspired air, (3) the content in the expired air, and (4) the concentration in the alveolar air. The first factor is multiplied by the difference between the second and third and gives (a) the amount of ethyl iodide absorbed per minute; the fourth factor is multiplied by the coefficient of solubility and this gives (b) the amount in the arterial blood; and (a) divided by (b) gives the volume of the blood-flow through the lungs. Improvements in the procedures for obtaining alveolar air or estimating its composition have also been effected. (The full data will be published in the *American Journal of Physiology*.)

The rate of conduction of nerve in the supernormal phase of recovery. By SYBIL COOPER.

Some experiments were undertaken in order to find out whether the rate of conduction of a nervous impulse is altered when the impulse travels in a nerve which is in the supernormal phase of recovery. This phase of recovery comes to a maximum (in the frog's sciatic) about

0.02 sec after the passage of an impulse, it is not always present but is most prominent after the nerve has been immersed in acid. During the supernormal phase there is an increase of excitability over the resting value and an increase of conductivity, *i.e.* the ability of an impulse to pass a block, but there is no evidence to show whether the second impulse travels at a greater rate.

The method used for determining the rate of conduction was that devised by Keith Lucas⁽¹⁾ in which the least interval for muscular summation is found at a point on the distal end of the nerve and is then found again when the first stimulus is at a known distance central from this point, this latter value is greater than the former, and the increase is due to the time of conduction from the central to the distal point. The Lucas pendulum was arranged to give three stimuli *A*, *B* and *C*. *A* was a maximum stimulus always applied at the central point, *B* was the first stimulus for determining the least interval and could be applied either at the central or distal point, and *C* was always applied at the distal point and was ten times the threshold strength at that point. Fluid electrodes were used and they were 2 cms apart. The rate of conduction was first found in the normal nerve in Ringer solution and then the fluid round the nerve was replaced by acetic acid in Ringer giving a *pH* of about 5. The nerve was left for a time until a good supernormal phase was present after a single shock. At 0.02 sec after a shock the excitability acquired a value about 120 p.c. greater than the resting value and with this strength of acid no decrement was set up in the nerve. The rate of conduction was found and then the stimuli for the measurement of the least interval were preceded at 0.02 sec by the stimulus *A*. To measure the least interval, *A + B + C* was required to sum over *A + C*. The rate of conduction was calculated in each case, and the results are shown in a table.

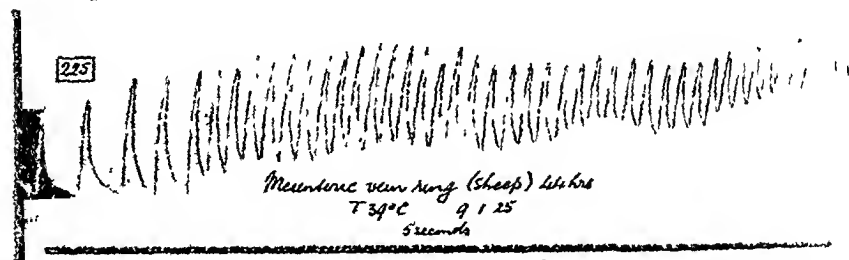
Expt	Temp °C	Nerve in Ringer		Nerve in Acid					
		Time for impulse to travel 2 cm sec	Rate metres/sec	Normal		In supernormal phase		Percent diff in rate	Percent increase of excita- bility
				Time for 2 cm	Rate	Time for 2 cm	Rate		
1	13.8	0.0080	23.1	0.0085	23.4	0.0086	22.7	97	—
2	14.5	0.008	25	0.0088	22.7	0.0088	22.7	100	123
3	13.8	0.008	25	0.008	25	0.0072	27.7	110.8	—
				0.008	25	0.008	25	100	130
4	12	0.0092	21.7	0.0084	23.8	0.0084	23.8	100	—
				0.01	20	0.006	20.8	104	—
				0.0088	22.7	0.0076	26.3	115	—
5	13.8	0.0088	22.7	0.0088	22.7	0.0088	22.7	100	120
				0.0072	27.7	0.008	25	90	—

The error on each value of the rate amounts to about ± 2 , and when the acid has been on the nerve some time, this error is increased as the nerve becomes fatigued and summation is much more difficult to detect. But the values obtained late in the experiments have been included in Exps. 3, 4 and 5, and the percentage difference in rate in all the experiments only amounts to 101.8. The percentage increase of excitability when measured accurately is shown above, but in all the experiments it had reached 120 p.c. before the rate was measured and there is no indication of a similar increase in the rate; therefore it may be concluded that when the nerve is not conducting with a decrement there is no alteration in the rate of conduction in the supernormal phase.

(1) Lucas. Journ. of Physiol. 46. p. 499. 1913.

The circulation in the mesenteric vein of the sheep.
(Preliminary Communication.) By K. J. FRANKLIN.

The investigation of the action of certain drugs on the isolated vein ring, begun in August 1924, has produced, *inter alia*, the results summarised below. These deal only with the vein in the absence of drugs, and are intended to provide a normal standard.



Mesenteric Vein Ring (Sheep) 44 hours after death. In Locke 6 parts, serum 1.
Largest contraction = diminution in lumen of 0.53.

The mesenteric vein ring of the sheep was selected from all the veins tried in three animals (sheep, ox, pig) as the one most suitable for a first series of experiments. Locke's solution without glucose was used for a long time, but in this solution the vein ring usually relaxed to a considerable extent. Occasionally during relaxation, or when in rare cases the vein retained its tone, rhythmic or arrhythmic movements were manifested. These were continued for from a few seconds up to three-quarters of an hour, and the largest individual contraction recorded would have meant in the intact animal a diminution of 0.15 in

the lumen of the vein. It was felt that if only some substance could be added to the Locke's solution to keep up the tone of the muscle, rhythmic movements would be able to commence and to continue for a longer period. At the suggestion of Dr M. H. MacKeith, the addition of serum was tried, and found to be fully justified. In most cases bloodstained sheep's serum has been added in the proportion of one in six. The results obtained since then have been sufficiently striking. The movements usually begin at once, and despite individual variations in size and regularity, are never entirely absent. The longest time during which they have been recorded is just over three hours: the largest contraction has been equivalent to a diminution of over half in the lumen of the vein. In one experiment in which the time taken by the upstroke in several contractions was compared with the total time for the complete movements, it was found to be about 0.2. Movements have been obtained up to three days after death. Finally, valves were looked up for in the veins, and several were demonstrable. They are of two kinds, the first consists of small ones over the entrance into the main vein of tributary veins; the second consists of paired valves, usually below the entrance of a tributary vein. There are vestigial remnants of valves in some veins, the valves proper having presumably atrophied, as is known to occur in human veins with increase in age.

It is suggested that the mesenteric veins in this animal act as an accessory heart in a system which is bounded by two sets of capillaries. The great power of contraction possessed by these veins, the relative speed of contraction as compared with relaxation, and the presence of valves, seem to afford justification for such a view.

In the calf's mesenteric vein ring rhythmic and arrhythmic contractions have been found.

The investigation is being continued.

I wish to thank Professor Gunn for the help which he has given and for the facilities afforded in his laboratory.

The asynchronism of the onset and end of right and left ventricular contraction. (*Preliminary Note*) By LOUIS N. KATZ

It is the current impression that the two ventricles begin and end their contraction simultaneously. The existing evidence however is not in accord with such an assumption. Thus it appears unlikely that the onset of contraction occurs synchronously in the two ventricles in view of the findings of Lewis⁽¹⁾ on the spread of excitation. Furthermore

it seems unlikely that both ventricles stop contracting at the same time when we realise that (1) the initial tension and volume of the ventricles as well as the arterial resistance against which they pump are different on the two sides and that (2) these two factors are the chief ones determining the duration of systole as shown by Wiggers and the writer(2). A direct investigation of this subject was therefore deemed advisable.

Pressure curves were recorded simultaneously and without parallax from the right and left ventricular chambers or from their aortas by means of the recently described optical manometers(3) and double-slit arc lamp(4).

An analysis of the curves so recorded showed that right and left ventricular contraction do not begin or end synchronously under normal experimental conditions, the asynchronism at the end being on the whole more marked than at the onset. As a rule the right ventricular contraction outlasted the left. These relations are altered by various changes in the experimental conditions. The asynchronism under normal experimental conditions is attributed to differences in spread of excitation on the two sides and in the duration of the two systoles. The changes in asynchronism are likewise attributed to variations in conduction below the common bundle and to independent changes in the duration of right and left contraction.

These findings have an important bearing on the interpretation of several cardiovascular phenomena. Probably the most significant of these is the relation of the asynchronism at the end of contraction to the T-wave in the electrocardiogram. Having shown that the contraction of the right ventricle outlasts the left and knowing that active muscle is electronegative to inactive muscle we can clearly appreciate how an electrical potential difference is set up again at the end of contraction and can produce a monophasic deflection. The variability in the asynchronism at the end of contraction and its reversal under certain conditions can explain respectively the variability in the appearance of the T-wave and its negative direction. We can also appreciate why the end of mechanical systole has such a variable relation to the T-wave.

(1) Lewis. *Arch. Int. Med.* 30. p. 269. 1922.

(2) Wiggers and Katz. *Am. J. Physiol.* 8. p. 439. 1922. Katz. *J. Lab. and Clin. Med.* 6. p. 291. 1921. Wiggers. *Am. J. Physiol.* 6. p. 439. 1921.

(3) Wiggers and Baker. *J. Lab. and Clin. Med.* 10. p. 54. 1924.

(4) Katz and Baker. *Ibid.* 10. p. 47. 1924.

The hydrogen ion concentration and oxidation-reduction potential of the cell-interior. By JOSEPH NEEDHAM and DOROTHY NEEDHAM.

Many of the earlier workers on intracellular oxidations attempted to discover the oxidising power of the cell by staining experiments using paraphenylenediamine and rongalit white. In the absence of any zero of oxidation-reduction potential to which these dyes could be referred, their experiments only showed that the dyes differed from one another and contributed little to our knowledge of the absolute oxidising power of protoplasm.

Recently, however, Mansfield Clark⁽¹⁾ has developed a system of oxidation-reduction indicators by the aid of which it is possible to determine the oxidation-reduction potential of a given system if the hydrogen ion concentration is known. He has introduced the symbol rH for reduction potential, rH being defined as the negative logarithm of the hypothetical hydrogen pressure in equilibrium with the oxidation-reduction system in question. The analogy of the symbol with pH is very marked, and just as buffer action refers to the stability of an acid-base system at approximately equal concentrations of acid and salt, so poisoning action refers to the stability of oxidation-reduction systems at approximately equal concentrations of oxidant and reductant. Both pH and rH are intensity factors.

We have employed the micro-injection method of Chambers⁽²⁾ modified, however, in certain details, and have studied the appearances caused by injecting 1-naphthol-2-sulphonic acid indophenol, one of the oxidation-reduction indicators into a strain of *Amæba proteus*. From the results of our experiments we conclude that the pH of the cytoplasm of *Amæba* is in the close neighbourhood of 7.6 and the rH is between 17 and 19. The latter value depends upon two assumptions, (a) that the oxidation-reduction system of the cell is of the type considered by Clark, and (b) that it is a poised system. The evidence for and against these assumptions will be discussed in detail elsewhere.

(1) Clark, W. M. U.S. Public Health Reports, Reprints Nos. 823, 826, 834, 848, 904, 915.

(2) Chambers, R. Anatomical Record, 24. 1. 1922.

Thrombocytes and blood coagulation. By J. W. PICKERING and H. GORDON REEVES.

The inception of blood coagulation is commonly regarded as due to the lysis of platelets. Achard and Agnaud⁽¹⁾ and Nolf⁽²⁾ have, how-

ever, maintained that the destruction of thrombocytes is the result of clotting. On this view, platelets do not participate in coagulation.

The following experiments were devised in order to test the value of these opinions.

Domestic fowl's blood was obtained through a carefully paraffined cannula from anæsthetised birds. It was shed into paraffined tubes externally cooled by ice and was then immediately centrifuged at 4000 revolutions per minute. Owing to the relatively large size of bird's thrombocytes, a plasma was obtained free from formed elements and their detritus. This fact was proved by microscopic examination of both unstained and stained samples. The platelets below the plasma were intact and not agglutinated. The plasma when removed from contact with thrombocytes exhibited the following characteristics:

i. It remained fluid on glass at 14–15° C. for five hours but was completely clotted in 24 hours. The clot remained unchanged for three weeks.

ii. It remained fluid for five hours, in clean glass vessels at 38° C. and thus differs from bird's whole blood which, under like conditions, clots in a few minutes(3).

iii. It maintained its fluidity after vigorous mechanical shaking in a sealed glass tube for 30 minutes, but clotted loosely after shaking for one hour.

iv. It clotted in 26 minutes 30 seconds on the addition of a feather, at 14° C.

v. On the addition of both bird's and mammalian serum it clotted immediately at 38° C.

vi. At room temperature (14–15° C.), the addition of centrifuged platelets caused clotting in 17 minutes 10 seconds. Coagulation was complete in 50 seconds at 40° C.

vii. It clotted at both room and body temperatures after oxalation and recalcification in 36 minutes and 8 minutes respectively.

The thrombocyte-free plasma of the bird, therefore, contains all the essentials for coagulation, but is much more stable than bird's whole blood. Thrombocytes though not absolutely essential for coagulation, are such great accelerators of the process that they must play an important part in arresting hæmorrhage.

(1) Achard and Agnaud. *Compt. rend. Soc. Biol.* 64. 716; 65. 549. 1908.

(2) Nolf. *Revue de Méd.* 30. 106. 1910.

(3) Pickering and Hewitt. *Biochem. J.* 15. 710. 1921.

PROCEEDINGS

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The effect of iodine on the metabolism of nitrogen and phosphorus in the growing pig. By F. C. KELLY.

Results obtained here in experimental studies of the dietary requirements for growth seem to indicate that the amount of iodine in the diet is an important factor and that a diet, consisting entirely or chiefly of cereals, may contain less than the optimum amount.

A series of experiments was therefore undertaken to determine the effect of addition of small quantities of iodine to a cereal diet, on the rate of absorption and retention of nitrogen and phosphorus in half-grown pigs, which, since weaning, had not received any iodine-rich food.

It was found that the addition to the diet used of amounts of potassium iodide varying in different experiments from 0.5 to 0.005 gm. per day, was accompanied by an immediate increased retention of nitrogen and phosphorus which was usually more marked in the case of nitrogen.

The figures in the following table are taken from the second of five experiments done, and show the effect of the addition of 0.25 gm. potassium iodide (0.19 gm. iodine) per day to the diet of a pig of 40 kilos receiving 1500 grms. of food daily. The ration consisted of oatmeal, maize, barley-meal, blood-meal and CaCO_3 .

For the sake of comparison, figures are given for a control animal to whose diet no potassium iodide was added.

Average amounts in grms. returned per day

	Experimental (KI added)		Control (no KI)	
	N.	P_2O_5	N.	P_2O_5
Pre-period 18 days	11 3662	4 7654	11 8649	4 6167
KI period 14 days	12 3049	5 4254	11-1897	4 8695
Post-period 14 days	11 4452	4 9911	11 0214	4-7397

These results point to the necessity for ensuring that a sufficiency of iodine is present in synthetic rations, which are presumed to contain sufficient of all the known essential constituents of food. This precaution, which apparently has not been taken in some experimental work

published in recent years, is of special importance when the physiological effects of unknown constituents of cod liver oil are being tested, as this oil is relatively very rich in iodine. Green food is usually also rich in iodine compared with other common foodstuffs, though weight for weight it contains only from one-hundredth to one-thousandth of the amount usually present in cod liver oil.

It is, of course, recognised that the well-known effect on nutrition of cod liver oil and green food cannot be attributed entirely to iodine, but it is suggested that when these substances are added to iodine-free rations the possible effect of their iodine content should be taken into consideration.

The supposed deficiency of prothrombin in hæmophilic blood.

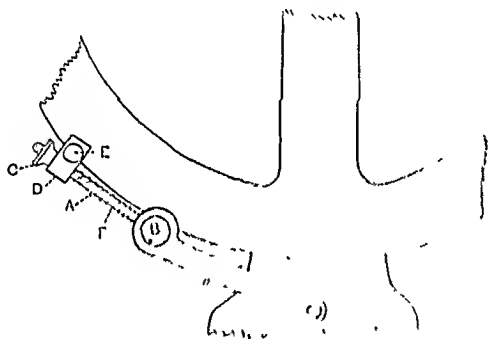
By J. W. PICKERING.

The suggestion that the delayed clotting of hæmophilic blood is due to a shortage of prothrombin has been recently revised and wrongly attributed to Addis(1), who rejected this hypothesis. It is based on the assumption that the slowness of clotting at room temperatures indicates a deficiency of prothrombin. No satisfactory method has hitherto been devised for comparing the production of thrombin in hæmophilic and normal bloods. In slow clotting shed blood partial inactivation of thrombin may be mistaken for a deficiency of thrombin. In the following experiments advantage was taken of the fact recorded by Pickering and Gladstone(2) that shed hæmophilic blood clots rapidly at 38° C. In Exp. 1, 1 c.c. of hæmophilic blood (with a clotting time of 48' 30" at 15° C.) was heated in a glass vessel to 38° C. It completely clotted in 6' 30". 0.5 c.c. of serum was pipetted off and added immediately to 10 c.c. of a solution of fibrinogen. At 15° C. the fibrinogen clotted in 3' 45". In Exp. 2, 1 c.c. of normal human blood was shed into a glass vessel with the temperature so regulated that it clotted in 6' 35". 0.5 c.c. of the serum formed was removed and immediately added to 10 c.c. of a solution of fibrinogen of the same concentration as that employed in Exp. 1. At 15° C. it clotted in 3' 42". It thus appears that the thrombin in hæmophilic blood is neither deficient in quantity nor altered in quality. It follows that the assumption of a shortage of its mother substance is unwarranted. These conclusions are consistent with the work of Addis(1) and with that of Wöhlisch(3). Addis(1) remarked that the slowness of the conversion of prothrombin into thrombin in hæmophilic blood remained unexplained. It becomes intelligible through the findings of

A micrometer attachment for the Keith Lucas pendulum. By HENRY DEXTER.

During the course of an experiment involving the use of the Keith Lucas Pendulum, it is usually necessary frequently to alter the distance between the two knock-out keys.

In the original instrument this necessitates the unscrewing of a flying clamp, the rotation of the wheel by hand and the tightening up of the clamp, for each adjustment made. When the alterations required are



of the magnitude of $0.1''$ of difference, it is sometimes difficult to make such changes accurately and at the same time conveniently.

In order to facilitate these adjustments I have evolved a micrometer attachment which enables the changes to be made expeditiously and with accuracy. The contrivance is very simple, and involves no structural alterations in the original instrument. The accompanying figure shows the essential features.

A is a brass rod 6 mm. diameter and 10 cm. long, with a boss, drilled out to fit accurately over the bolt, and between the jaws, of the original clamp *B*. The free end of this rod is screwed for a distance of 25 mm. upon which screw, works a knurled nut *C*. Sliding on the rod is a heavy brass block *D*, 30 mm. long, 14 mm. broad and 16 mm. thick, with a slot in its upper half wide enough to allow the edge of the wheel of the pendulum to be inserted. This block is clamped to the wheel by means of the binding screw *E*, and is retained hard against the nut *C* by means of the compression spring *F*.

In use the rough adjustment of the knock-out keys is made in the usual manner, utilising the standard clamp. During this procedure the "micrometer" is hanging down out of action. When the setting of the keys has been found to within $\pm 2^\circ$, the attachment is then brought up and the block *D* clamped to the wheel. The screw of the original clamp is loosened and left loose for the rest of the experiment. The adjustments to the vernier scale are finally made by now turning nut *C*.

The attachment is smooth and accurate in action, and perfect adjustment is readily and rapidly obtained, the spring *F* obviating any backlash.

Chloroform rigor in frog's muscle. By A. D. RITCHIE.

Rigor mortis consists of an irreversible change in muscle whereby it becomes (1) shorter, (2) opaque, (3) different in elastic properties, and usually (4) acid. These changes can be brought about artificially by various means such as the action of chloroform and ether, or by means of acids, and can occur spontaneously after death. It is not necessary to assume that the mechanism of the change is the same in all cases.

If a frog be perfused through the aorta with ice-cold Ringer's solution containing neutral red and subsequently with Ringer's solution saturated with chloroform, rigor can be observed to set in prior to any change in reaction of the muscles, as indicated by the colour of the neutral red. By perfusion with Ringer containing a trace of ammonia the tissue reaction can be made alkaline and the effect is more striking as many muscles will be found in rigor while they are still definitely alkaline. After the onset of rigor there is a considerable development of acid. It is clear, however, that the immediate effect of the chloroform in producing rigor cannot be due to the acid.

On treating living fibres of frog's muscle on the stage of the microscope with chloroform-Ringer, it is seen that the chloroform produces

a more or less complete breakdown of the structure of the muscle fibre, in which the cross striation becomes obliterated and the outline swollen and irregular. At the same time minute globules of high refractive index which stain with osmic acid usually make their appearance. It is suggested that rigor, at any rate that form produced by chloroform, is due to a disturbance of the surface relations of the fatty and aqueous constituents producing a breakdown of their normal arrangement. This conclusion may be compared with the observations of Lapicque and Legendre (1) on the action of anæsthetics on the myelin sheath of nerve fibres.

(1) *J. de Phys. et de Path. Gén.* 20. p. 163. 1922.

Microscopical observations on the cerebral circulation.

By HOWARD FLOREY.

Cats, rabbits, and monkeys were the animals used and the cortex was exposed by trephining in the parietal region of the skull.

A binocular dissecting microscope was employed, the maximum magnification obtainable being 100 diameters. A 100 c.p. Pointolite was the source of light.

By this means a good view of the circulation could be obtained, the arteries and veins being easily distinguished, and the flow in the capillaries and small veins quite visible.

The arteries and the arterial ends of the capillaries reacted by a very vigorous contraction to a mechanical stimulus. Sometimes to weak mechanical stimuli the reaction was that of a slight dilatation. Electrical stimuli applied by means of a stigmatic electrode were found to produce the same contraction; sometimes with a strong stimulus a varicose condition was set up.

A temperature of 45° C. was found to produce a contraction of the arteries, one of 40° C. dilatation.

Ice-coldness produced a contraction of the arteries.

To these various stimuli the arteries show differences of sensibility, some contracting completely, others being scarcely affected.

Adrenalin was applied to the cortex, the floor of the fourth ventricle, and the anterior surface of the medulla. In no case was contraction produced, but occasionally a slight dilatation appeared. No constrictive effects were noticed when the adrenalin was injected intravenously.

Pituitrin produced no effect. Barium chloride in very strong solution produced contraction, nitrates and strychnine dilatation.

The question of the innervation of the cerebral vessels was investigated.

The vaso-motor centre was directly stimulated without effect. The stimulation of the stellate ganglion and the cervical sympathetic cord also had no influence on the calibre of the cerebral vessels. Asphyxia, in which there is active stimulation of the vaso-motor centre, and thujone convulsions in which the same thing occurs, were found to be unaccompanied by active changes in the calibre of the cerebral vessels.

The conclusion drawn is that there is no vaso-motor control of the cerebral vessels.

Inflammation was also studied. In spite of the fact that there are no nerves the changes observed are similar to those occurring in other regions, except that there is a tendency for the inflammation to be limited approximately to the point which is irritated, which fact is interpreted as evidence of the lack of vaso-dilators with their corresponding axon reflex.

Thrombus formation can be well observed in the vessels of rabbits.

The effect of insulin on blood volume. By H. D. KAY and W. SMITH.

As mentioned in a previous paper¹ different preparations of insulin produce diverse effects on the blood volume of rabbits as measured by the hæmoglobin percentage. It has since been found that subcutaneous injection of the insulin hydrochloride now on the market, prepared by Dodd's process, brings about a considerably smaller increase in blood volume than that produced by the cruder kinds used in the earlier experiments. With one of these purer specimens the fall in the percentage of hæmoglobin is only 7-8 p.c., whilst control animals given the same volume of saline show a fall of 3-4 p.c. during the same period of time. With some animals there is an initial increase in the hæmoglobin value above 100 p.c., usually in the first 60-90 minutes after the injection, followed by a fall to about 92 p.c. of the original value. Another specimen of insulin hydrochloride has a still less effect on the hæmoglobin percentage. In all the cases examined recovery from convulsions was effected by injections of glucose. The recovery was accompanied by a rise in the hæmoglobin value to over 100 p.c. of that originally present—an apparent diminution in the blood volume.

The effect on the hæmoglobin percentage of certain fractions obtained during the preparation of crude insulin by Dudley's method² has been

¹ Haldane, Kay and Smith. *This Journ.* 59. p. 193. 1924.

² Dudley. *Biochem. Journ.* 17. p. 376. 1923.

examined in order to determine if the substance which produces the blood volume effect (apparently not the same factor as insulin) is concentrated in any one fraction. The solid matter found adhering to the bottom of the vessel in which the final aqueous solution has been made up to 80 p.c. with alcohol and allowed to stand overnight (salt fraction), was separated and injected in doses of 30 mg. to 2 kg. rabbits. This fraction gave on the average a fall in the hæmoglobin percentage from 100 to 88 in four hours, at the same time the blood sugar fell from 0.10 p.c. to 0.07 p.c. The "yellow fraction" (the yellow syrup which also separates at the stage just mentioned) gave varying results. Of eight animals examined, four gave a distinct rise in the hæmoglobin percentage, whilst four gave a slight fall. Compared with control animals there was a rise of 4 p.c. in the hæmoglobin at the end of three hours. The effect of the yellow fraction on the blood sugar was also variable, in some cases a rise was noticed. This rise was not always, however, accompanied by a rise in the hæmoglobin.

It would appear, therefore, that in rabbits' blood dilution is not a specific insulin effect.

Erratum.

The author of the Communication entitled 'The Hooding of Birds,' Proceedings, Nov. 15, 1924, is Dr D. Patrick and not W. Carlier as stated in the Proceedings.

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